406 MEDICAL GENERAL LABORATORY

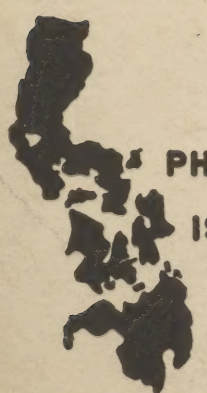
ANNUAL HISTORICAL REPORT

1949

TOKYO, HONSHU, JAPAN

• OKINAWA

• IWO JIMA



PHILIPPINE ISLANDS

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PROFESSIONAL SECTION

PREFACE

Following the precedent set in 1948 the Annual Historical Report of this unit has been divided into two sections. An Administrative Section previously submitted pertains to operational activities of primary importance to various superior headquarters of the Medical Department of the Army. The Professional Section records activities of this unit in fulfilling its mission to supplement the epidemiologic, sanitary, and diagnostic services available in other medical department laboratories and to investigate outbreaks of disease and conditions which affect, or may affect the health of persons or animals of the command.

Investigative work has served the purpose of continuing a broad-scale post-war program of Medical Department Research, in this respect making some contribution to medical knowledge concerning those diseases peculiar to the Far East. Such efforts have served at the same time as an excellent opportunity for training personnel of the Armed Services in special aspects of laboratory work.

The cooperation offered by military commanders, their medical advisers, and all other echelons of Medical Department activity of Army, Navy, and Air Force was largely responsible for the material allowing the studies recorded herein. Appreciation is also recorded for the guidance, recommendations, and support freely furnished by the Pathology and Allied Science Division of the Surgeon General's Office, the Army Medical Department Research and Graduate School, and the Virus Commission of the Armed Forces Epidemiologic Board.

Inasmuch as a large portion of the investigative work has been concerned with native populations, close cooperation has been necessary with the Japanese National Institute of Health through supervision and guidance of the Public Health and Welfare Section, SCAP, including the prefectural health authorities and medical institutions, particularly those of Tokyo, Okayama, and Yamanashi. Acknowledgement is also made of cooperation afforded by the Ministry of Health and the Military Advisory Group of Korea.

Thanks are also due the medical supply sections of Eighth Army and GHQ, the Fifth Medical Depot, the Third Military Railway Service, and various other agencies for their demonstrated willingness to support us in our numerous requests.

Specific mention is made of the organizational ability of the previous Commanding Officer under whom most of the work was accomplished during 1949. This ability is also reflected in the relative ease with which overall programs have been continued despite numerous other personnel losses.

Again it is emphasized that the activities and their compilation is a reflection of the earnest cooperation of the individual officers, civilians, and enlisted men with the assistance, technical and otherwise, of our complement of Japanese, Chinese, Korean, Viet Namese, and Stateless employees.

R.L.H.

CONTENTS

MEDICAL ZOOLOGY

The work accomplished by the Section of Medical Zoology during 1949 may be divided into the following categories: routine stool examinations on certain groups of Japanese Nationals employed by the Army; routine stool examinations on Occupation personnel; special tests; research projects; training activities of officers, enlisted men, and Foreign and Japanese National personnel; preservation and distribution of parasitic materials and specimens for research and teaching purposes.

A summary of all stool examinations made during 1949 appears in Table I. A total of 13,203 different specimens were examined, including a total of 20,414 different microscopic preparations.

Table I. Summary of Stool Specimens Examined During 1949

	Number of Specimens	No. of Microscopic Examinations
Routine stool examinations (Japanese)	2,258	2,320
Routine stool examinations (American)	3,613	3,874
Dilution egg count project		
Number examined by MGL and AMS	529	1,058
Number examined by dilution egg count technique (2 microscopies each)	313	626
Stools examined during epidemiological surveys		
On Island of Shikoku	1,720	3,472
On Island of Okinawa		
Natives 2,145		
Americans 340		
Filipinos 11		
Total 2,496	2,496	5,003
On Island of Hokkaido	2,212	4,515
Grand Total	13,203	20,414

Routine

ROUTINE STOOL EXAMINATIONS ON JAPANESE AND AMERICAN PERSONNEL: A total of 2,258 Japanese Nationals from the Tokyo-Yokohama area employed as food handlers or servants were examined routinely for intestinal parasites. Of this number, 74.3 percent were parasitized; 67.6 per cent harbored helminths, and 26.1 per cent had protozoan infections. Only 2.5 per cent had E. histolytica. A comparison with 2,655 Americans examined in a similar manner is shown in Table II.

TESTS FOR URINARY GONADOTROPINS: During November and December, 1948, 12 initial tests were run using the Xenopus laevis female (South African clawed toad) as a test animal for the determination of chorionic gonadotropins. From January through July 1949, this animal was depended upon for determining pregnancy. In August it was decided to test the male frog (1), Rana sp., that could be secured locally. The two amphibians were used in parallel series in an attempt to determine their relative efficiency as test animals. To date 99 Rana have been run on urines which proved to be negative with Xenopus and 50 on urines positive with Xenopus (Table III).

Early in the project, tests with the Rana were run using fresh (unconcentrated) urines and 8 of 15 test animals died, whereas no deaths have occurred when a concentrated inoculum, the same as that prepared for the Xenopus test animals (1) was used. Since this observation was in agreement with that of Cutler (2), the use of fresh urines was discontinued.

Of 50 male Rana which were tested with urines found positive by Xenopus, three gave negative results. A total of 99 Rana were tested with urines found negative by Xenopus, and of this number, 96 were negative and three positive. Follow-up and correlation with clinical findings are still incomplete.

Table II. Incidence of Parasitism in Americans and Japanese Examined Routinely

	Americans*		Japanese	
	Number	% Infected	Number	% Infected
No. persons examined	2,655		2,258	
No. parasitized	869	32.7	1,677	74.3
No. with helminths	339	12.8	1,527	67.6
No. with protozoa	618	23.3	589	26.1
Helminths:				
Ascaris	209	7.9	1,018	45.1
Hookworm	70	2.6	283	12.5
Whipworm	72	2.7	576	25.5
Trichostrongylus sp.	16	0.6	336	14.9
Strongyloides stercoralis	8	0.3		
Clonorchis sinensis	1	0.03	21	0.9
Metagonimus yokogawai	1	0.03	2	0.1
Hymenolepis nana	2	0.1	1	0.04
Enterobius vermicularis	1	0.03	3	0.1
Taenia sp.			2	0.1
Hymenolepis diminuta			1	0.04
Schistosoma japonicum			10	0.4
Protozoa:				
Endamoeba histolytica	97	3.7	56	2.5
E. coli	269	10.1	355	15.7
Endolimax nana	303	11.4	271	12.0
Iodamoeba butschlii	30	1.1	15	0.7
Giardia lamblia	99	3.7	59	2.6
Chilomastix mesnili	11	0.4	5	0.2
Trichomonas hominis	1	0.03		
Enteromonas hominis	8	0.3		
Dientamoeba fragilis	1	0.03		

* Japanese wives of American personnel and Foreign Nationals are classed as Occupation Personnel in this table.

Table III. Tests for Urinary Gonatropins Using Female Xenopus laevis and Male Rana

	No. Rana tested and Results		
	No. Positive	No. Negative	Total Comparison
Urines Positive with Xenopus	47	3	50
Urines Negative with Xenopus	3	96	99
Total Rana test animals used			149

During 1949, a total of 419 urines were tested for pregnancy and 25 for neoplasms in the human male.

MISCELLANEOUS IDENTIFICATIONS: A limited number of blood specimens have been received for the diagnosis of malaria as well as filariasis and sputum for parasitic ova. In addition, various entomological and other specimens were submitted for identification (Table IV).

Research

STUDIES ON SCHISTOSOMIASIS: Since July 1947 a continuous program has existed aimed at collection of basic facts, and study of methods of improved diagnosis and control of Schistosomiasis japonicum. While considerable progress was made in both 1948 and 1949 this account must still be regarded in the nature of a progress report. Earlier findings are recorded in previous annual reports of this laboratory.

Table IV. Summary of Miscellaneous Identifications

Blood Specimens		Dog Stool	2
For malaria	16	Rabbit Stool	2
For filariasis	2	Insects for identification	5
Human stool for tapeworm	2	Larvae for identification	2
Human stool (Scolex)	1	Fish for identification	1
Rectal swab	1	Snakes for identification	2
Adult helminths	8	Earth for ova	1
Urine	1	Surgical Tissue Sections	2
Sputum	3		

LABORATORY SCREENING TESTS FOR MOLLUSCICIDES: During 1948 and early 1949 the efficacy of various molluscicides was tested by placing the snails directly into solutions of various concentrations of chemicals. The number of snails that survived in a given period of time was taken as a rough index of the efficiency of the chemical as a molluscicide in the dilution used. These results determined whether or not the chemical should be given a field plot test.

In the spring of 1949 a new method (3) of exposing snails to preliminary screening tests was adopted. Briefly the procedure involved placing the snails in petri dishes on filter paper saturated with the dilution of the chemical being tested. The paper might be dry or moistened. Deaths occurring within the four day test period were considered indicative of molluscicidal action since control snails survived well on moist, untreated filter paper. Consequently, a number of chemicals previously tested by the original method were retested by the petri dish plate method. Several of these warrant further study and tests as potential molluscicides. The results are summarized in Table V.

Table V. Chemicals Used for Laboratory Tests in Snail Control

Chemical	Source	50% end point secured with dilution of
Ammonia	-	1:1000
Parathion-Thiophos 3422 15% wettable powder	American Cyanamid Co.	1:4000
Aroclor 1232 - dry	Monsanto	1:3000
Aroclor 1232 - wet	Monsanto	1:23,255
Aroclor 1242 - dry	Monsanto	1:3000
Aroclor 1242 - wet	Monsanto	1:23,255
Aroclor 1248 - dry	Monsanto	1:3000
Aroclor 1248 - wet	Monsanto	1:23,255
Chloronitro-biphenyl - dry	-	1:3000
Chloronitro-biphenyl - wet	-	1:23,250
Benzene hexachloride, delta isomer-	Dow	
xylol dissolved then dried moist-		1:18,000
ening snails		1:80,000
Pentachlorophenol	Dow	
-dissolved in alcohol		1:25,328
-dissolved in xylol and dried		1:68,550
-xylol solution applied wet		1:80,000

Essentially Negative Results were Secured with the Following

Copper sulfate	-	1:1000
Zinc sulfate	-	1:1000
Compound 118 - dry and wet	Julius Hyman Co.	-
"Hepta-Klor" - dry and wet	Julius Hyman Co.	-
Compound 497	Julius Hyman Co.	-
DDT		
Methoxy analog of DDT		
Lethane 384	Rohm and Haas	
Rothane	Rohm and Haas	

FIELD CONTROL EXPERIMENTS IN YAMANASHI PREFECTURE: When indicated by laboratory screening tests chemicals were used in "field plot tests", and finally, three of the most promising ones were obtained in quantities to carry out field control experiments. The three chemicals tested were: (1) DN-111 (20% dicyclohexylamine salt of dinitro-op cyclohexylphenol) Dow Company, (2) DN No. 1 (40% dinitro-o-cyclohexylphenol) Dow Company and (3) Santobrite (H.S.P.A. activator) (79% sodium pentachlorophenate) Monsanto Company. This latter phase of the work started in the fall of 1948, was continued during 1949, while final evaluations still await observations to be made in 1950.

A total of 63,600 linear feet of irrigation ditch were treated in the fall of 1948 and 35,405 additional feet in the spring of 1949, thus furnishing a basis for a comparison of the two seasonal periods when snail control is feasible. Those areas treated in the fall were re-treated in the spring, thus obtaining not merely the advantage of two treatments but also the combined control advantages which may be afforded by the two seasons. The various localities where the control work was carried out have been described (3). Their selection was based on certain definite criteria. For example, some were essentially islands between river branches with little or no chance of repopulation from the outside, while others were situated with snails in the watershed above them. Various kinds of ditch conditions were selected: clear or dense with vegetation, muddy, stony, rock or cement-lined, and ditches with culverts or bridges were included. The areas also represented varying degrees of wetness and amounts of running water.

Snail counts were made in numerous foot-quadrat units prior to treatment in each of the several control areas. Equivalent post-treatment quadrat counts of live snails made at the end of 3-4 weeks, and either 2½ or 5 months, served as a basis for computing the percentage of reduction in snail population; if the post-treatment count was five and the pre-treatment 100, the population reduction would be 95 per cent. The increase in the proportion of dead snails occurring in the collections after treatment was also obtained, but such figures were considered inadequate as an evaluation of molluscicidal action, since living snails are more conspicuous and would be picked up in disproportionate numbers.

Population reductions in percentages are shown in Table VI for individual control areas treated during either the fall or spring, and those treated both times. The chemical used in each instance is also indicated.

The average population reduction for all the areas treated in the fall was 67.6 per cent at the end of 3-4 weeks and 81.7 per cent after 5 months. In contrast to this, the reduction 2½ months after the spring treatment was no greater than after 3-4 weeks, both in areas previously untreated, and, also, those which were treated the previous fall. It seems unlikely that a differential would have been found at five months after the spring applications. In fact, the picture might have been confused somewhat by snail migration and reproduction during the summer. The long period of hibernation following exposure to the chemicals in the fall may explain this difference in the fall and spring trials. If this is the case, higher initial and more consistent kills in the fall, such as those obtained in the spring, should be sought. Then, if hibernation is critical, a very high rate of kill might be expected after the winter months. The spring figures for population reduction are higher at the end of 3-4 weeks than the fall figures are after 5 months.

Field conditions, farming procedures and snail habits make chemical control feasible for only short periods during the spring and fall months. Varying conditions associated with these two seasons make it advisable to note differences in the data which might give preference to one or the other. For spring applications collectively, a population reduction of 87.8 per cent was obtained, and the corresponding figure for fall was 81.7 per cent. This is a difference warranting consideration were it not for the fact that the lesser fall figure reflects low rates of kill in two villages, Toyotomi and Kagami-nakajo, where conditions were unfavorable to chemical application. At the former place the chemical was put on dry and there was no rain for eleven days, allowing chemical dissipation and accounting for snail protection through dilution. Further, the facts should not be overlooked that in these two areas only small numbers of snails were involved, which is not reflected because percentages have been averaged to show the average per cent for several areas. Yet ultimate success requires good kills whether snail colonies are small or large. Likewise, interference to molluscicidal action also occurred in at least two areas treated in the spring, but with lesser influence on snail reduction - Hitotsuya and Tanooka #2.

Table VI. Comparison of Results Obtained Following Fall and/or Spring Applications Of Chemicals DN-III, DN-I and Santobrite in Yamanashi Prefecture

Areas	Pre-Treat. Nos. of Live Snails	FALL APPLICATION Pop. Reduction After: 3-4 wks.(%)	5 mos.(%)	SPRING APPLICATION Pop. Reduction After: 3-4 wks.(%)	2½ mos.(%)	FALL & SPRING APPLICATION Pop. Reducation After: 3-4 wks.(%)	2½ mos.(%)
			CHEMICAL DN-III				
Toyotomi	35	54.3	42.9			100.0	94.3
Tanooka #1	1,594	64.2	89.6			98.6	98.4
Chiyoda	173			87.9	90.1		
Average		59.3	66.3	87.9	90.1	99.3	96.4
			CHEMICAL DN-I				
Kagami-Nakajo	70	68.6	66.6			98.6	92.9
Tatsuoka	591	89.8	90.2			95.3	93.9
Tomi #1	805	75.9	96.0			99.6	98.4
Tomi #2	387			97.1	90.7		
Kasugai	758			93.1	95.7		
Asari	105			94.3	89.5		
Average		78.1	84.3	94.8	92.0	97.8	95.1
			CHEMICAL SANTOBRITE				
Nirasaki	1,362	39.5	89.0			94.2	92.1
Mikage	272	80.9	97.8			97.1	97.8
Hitosuya	854			80.6	91.0		
Tanooka #2	472			73.1	68.6		
Minamoto	45			93.3	88.9		
Average		60.2	93.4	82.3	82.8	95.7	95.0
Average for all chemicals		67.6	81.7	88.5	87.8	97.6	95.5

On the basis of these data it seems questionable whether one season should be designated as preferable to the other. Combined fall and spring applications extended the reduction in snails, but did not result in eradication. As is true of all pest control work, the last few per cent of the population is difficult to eliminate.

The relative efficacy of the three chemicals must necessarily be considered. Considering fall applications only, the snail reduction obtained by the three chemicals is as follows: DN-III, 66.3 percent; DN-I, 84.3 percent; and Santobrite, 93.4 percent. Here again, if the limiting field and climatic conditions at Toyotomi and Kagami-nakajo are taken into consideration, the differences would be greatly reduced. A comparison of the findings for spring applications is different: DN-III, 90.1 per cent (single area); DN-I, 92 percent; and Santobrite, 82.8 percent. Conditions surrounding the application of Santobrite could readily have accounted for its poorer showing in the spring. At Hitotsuya and Tanooka #2, some rather populous colonies remained; a rain occurred immediately after application at the former and water was turned into the ditches shortly after treatment at the latter place.

The data available gives no clear cut evidence that either one of the three chemicals is markedly superior to the others. On the basis of fall figures, DN-III was inferior and the improved results in the spring were based on a single area. Estimates of cost for DN-I and Santobrite are about the same. The latter as dust is irritating to mucous membranes, but this is not a serious matter when the chemical is applied in solution.

The snail collections made in July, 2½ months after spring treatments, did not include young snails, but they did appear in limited collections made in September, approximately 5 months after chemical application.

Summary: The three chemicals applied in field tests proved to be good molluscicides, whether applied in the fall or spring, and the snail population was reduced to a very

low level in areas treated during both fall and spring seasons. However, evidence of repopulation was seen even at the end of the first summer following treatment. There was little evidence of a residual action on the part of either chemical.

SEASONAL CYCLE OF S. JAPONICUM INFECTIONS IN THE INTERMEDIATE HOST IN YAMANASHI PREFECTURE AND SAGA-FUKUOKA PREFECTURES (KYUSHU): Since the fall of 1947 monthly collections of about 500 snails have been made at a series of permanent stations near Kofu, Yamanashi Prefecture. In July 1949 the project was extended to include collections from the endemic schistosomiasis center in Kyushu. The snails are first measured to determine their maturity, and then dissected in order to recognize the incidence of infection and the approximate age of each infection. From the data collected it has been possible to determine the time of year snails are most apt to acquire their infections, principle shedding periods of the cercariae, length of time that the snail infections persist, condition and fate of infections during hibernation and the relationship of the infections to agricultural activities. Also numerous observations on the ecology of the snail have been made as the result of repeated collections. The above points have been previously discussed (4).

During the year 1949, a total of 27,429 snails were collected and examined from Yamanashi Prefecture (Table VII). Of this number 590, or 2.2 percent, were infected, the variation in monthly incidence ranging from about 1-3 percent. A total of 6,170 Kyushu snails were examined from July through November, and 470 or 7.6 percent were infected. The monthly incidence varied from 6-10 percent during these months (Table VII).

Table VII. Summary of Data Obtained in the Monthly Examination of Snails, Oncomelania nosophora, from Yamanashi Prefecture (1949)

Month	Number of Snails	Number of Infections	Incidence of Infection	Average Age of Infection (Weeks)
January	2,210	56	2.4	15.4
February	2,191	64	2.9	13.2
March	2,359	50	2.1	15.2
April	2,398	59	2.5	15.9
May	2,210	45	2.0	12.9
June	2,356	53	2.2	14.2
July	2,303	71	3.1	12.3
August	2,090	51	2.5	16.5
September	2,247	21	0.9	16.2
October	2,448	40	1.6	15.8
November	2,496	29	1.2	15.9
December	2,121	51	2.4	13.2
Totals	27,429	590	2.2	-

FROM SAGA AND FUKUOKA PREFECTURE, KYUSHU (1949)

Month	Number of Snails	Number of Infections	Incidence of Infection	Average Age of Infection (Weeks)
July	1,806	133	7.4	15.5
August	670	57	8.5	17.6
September	1,534	93	6.1	16.4
October	1,069	80	7.5	15.0
November	1,091	107	9.8	15.2
Totals	6,170	470	7.6	-

In Figure I and Table VIII the incidence of infection and the relative frequency of mature, young mature, and immature infections is shown for the total monthly collections in Yamanashi Prefecture, both for 1948 and 1949. In July 1948 there was a marked increase in the incidence, due largely to immature stages. During the same period the following year (1949) there was only a slight increase in the occurrence of infections, less

than one per cent. Although there was no peak during the summer of 1949, a noticeable drop in incidence occurred during September, October and November, which corroborates the previous report that snails remain infected only for a relatively short period of time in the summer months.

In the fall of 1948 (October and November), there was a moderate increase in the incidence of infections. This rise was not duplicated in 1949 until December, when a noticeable number of young infections occurred at one of the four collecting stations, Mutsusawa.

The snails went into hibernation with a lower incidence of infection in 1949 than they did in 1948 and for both years mature infections were in predominance; in Kyushu mature and young mature outnumbered immature over three to one in November (Figure 1).

At Yamanashi stations collectively the incidence and maturity of infection did not fluctuate noticeably throughout the entire year and this was essentially true in Kyushu for the months of July through November.

Table VIII. Summary of S. japonicum Infections in Oncomelania nosophora, Yamanashi, 1949

Month	Number of Infections by Age Group*									Totals
	a	b	c	d	e	f	g	h	i	
January	1	0	3	3	0	2	13	34	0	56
February	0	0	5	5	4	3	20	27	0	64
March	0	7	3	4	4	0	1	29	2	50
April	1	3	5	3	2	1	4	39	1	59
May	0	1	2	8	9	4	0	20	1	45
June	0	3	7	7	6	3	2	21	4	53
July	0	9	4	11	7	5	5	29	1	71
August	0	0	1	1	0	8	9	30	2	51
September	0	0	1	2	2	0	1	15	0	21
October	0	0	1	5	4	0	3	27	0	40
November	0	0	2	3	2	0	2	20	0	29
December	1	6	3	3	5	2	5	26	0	51

* a - 2 weeks
b - 3 weeks
c - 4-5 weeks
d - 6 weeks
e - 7 weeks
f - 8 weeks
g - 10 weeks
h - 20 weeks
i - 40 weeks

These ages are based on the appearance of the infection and the time required for development to reach the stage present under optimum conditions. Immature includes up to 8 weeks; young mature up to 10 weeks and mature infections include all others. (Adapted from Faust and Melaney (6).

A comparison of 1948 and 1949 monthly data for the individual collecting stations warrants consideration (Figures 2 and 3). At Aburakawa (Ido) the findings for the two years are somewhat similar, except that the increase of infections in the fall of 1949 was delayed until December. This may be fortuitous and related to a break in population homogeneity. The reason for this opinion is that December infections were predominantly mature and there were no young infections in October and November to account for them. For the rise to be valid, in light of the findings for the previous two months, the infection should have been very immature. Actually, deviations in the collecting site occurred in September and November due to disappearance of snails. Although no more than ten meters intervened between old and new sites, the nature of the data suggests that there was a lack of homogeneity in the snail infections at the three sites.

FIGURE I.

SEASONAL STUDIES OF S. JAPONICUM IN THE SNAIL.

TOTAL COLLECTIONS.

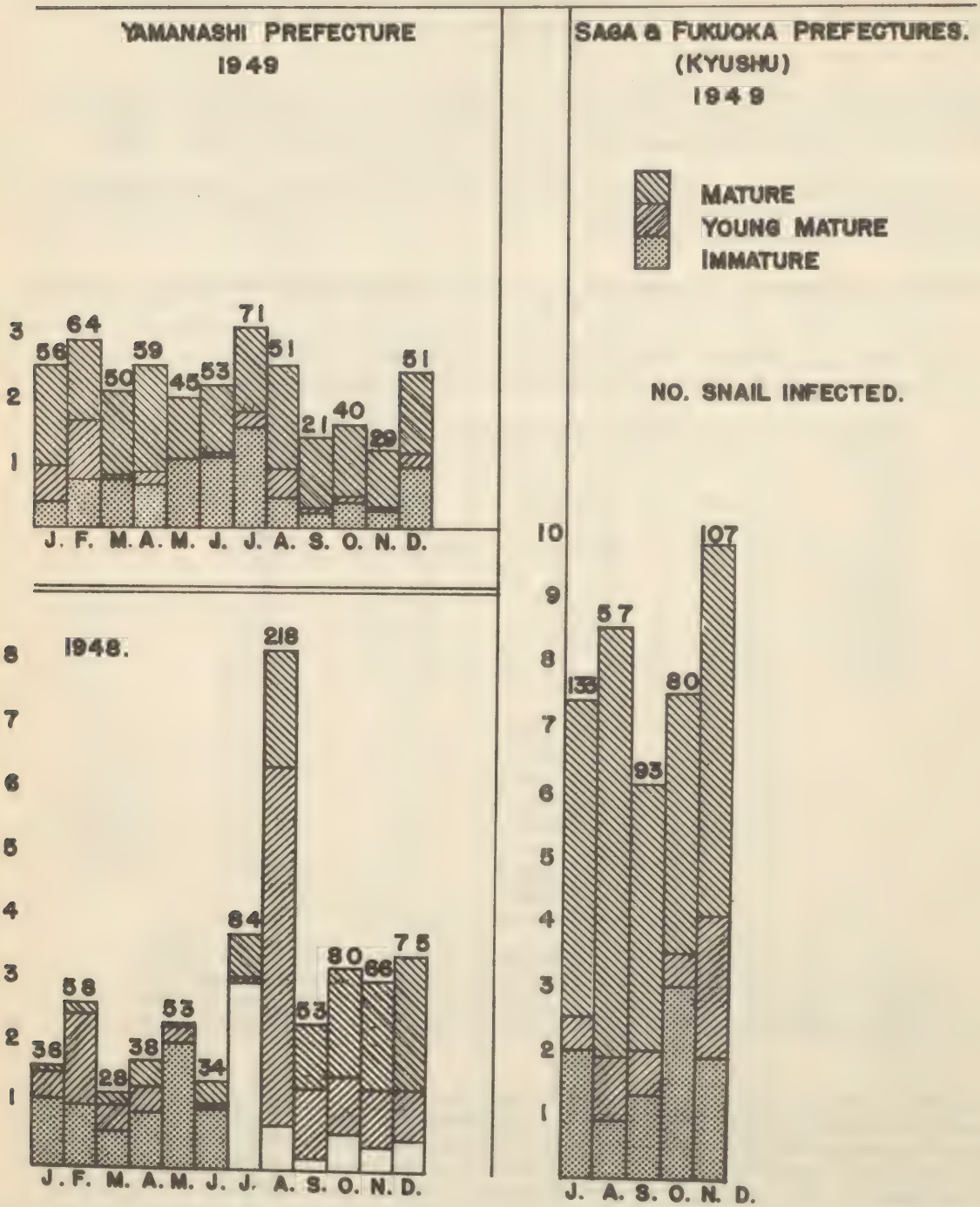


FIGURE 2.

SEASONAL STUDIES OF S.JAPONICUM IN THE SNAIL.
YAMANASHI PREFECTURE.

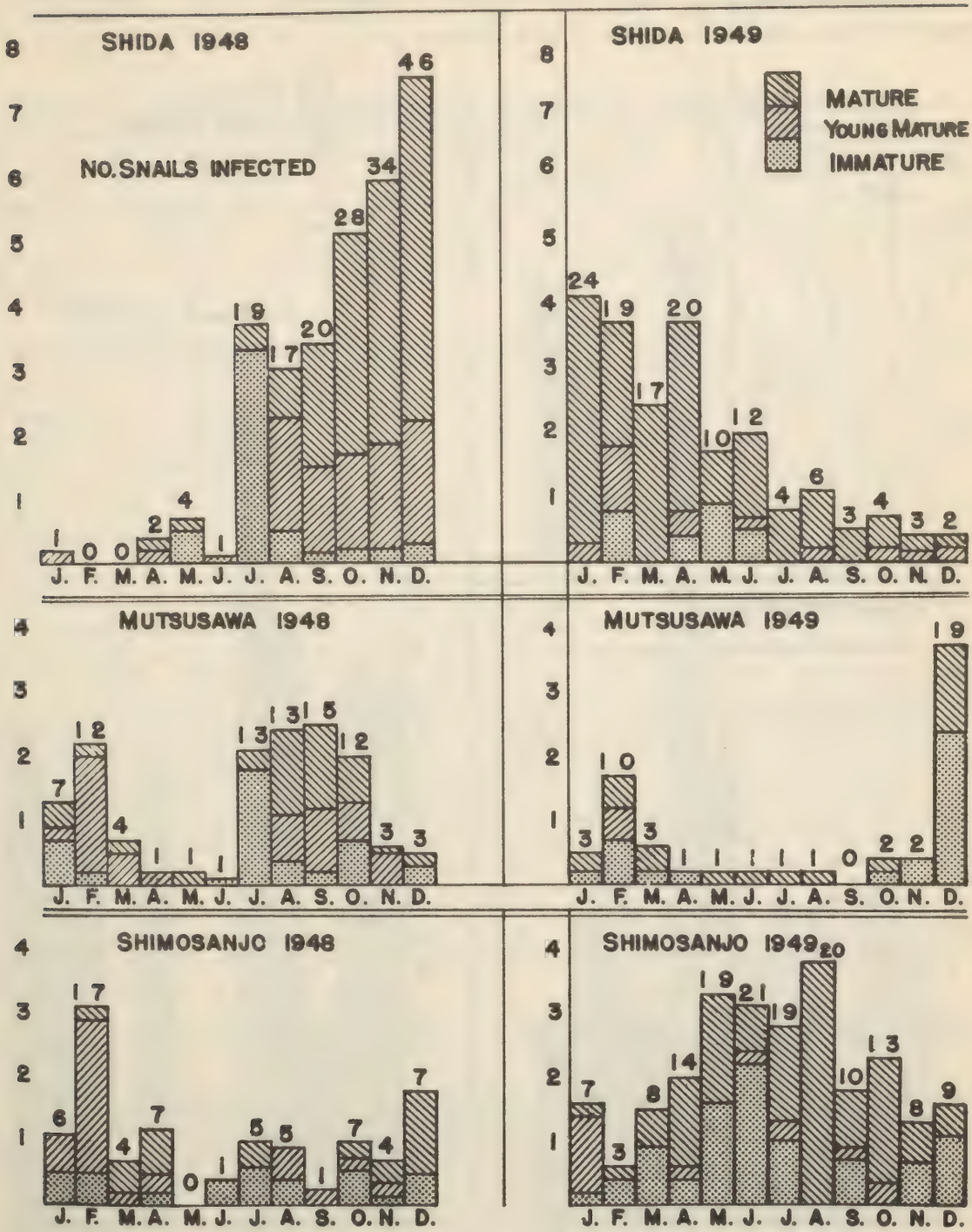
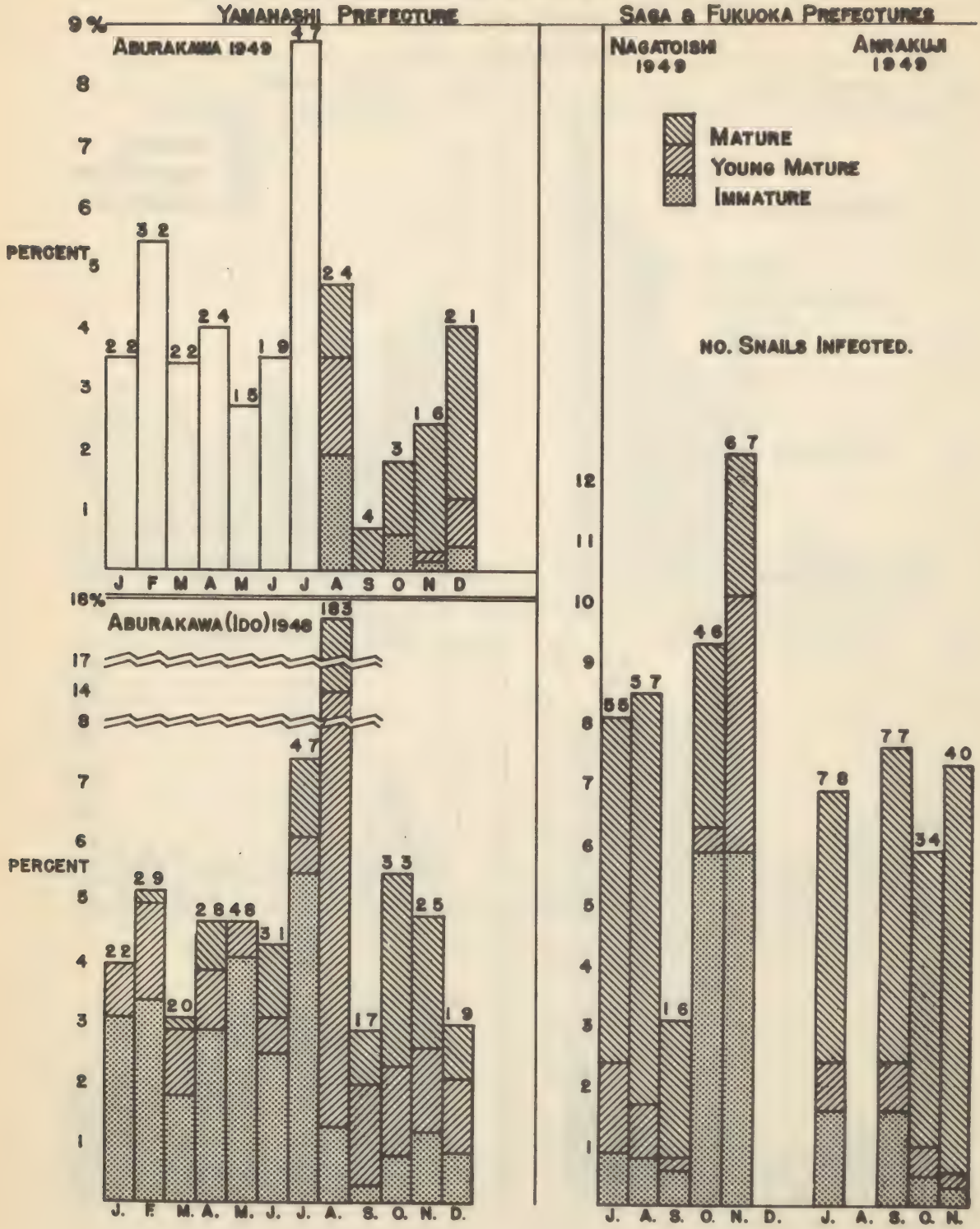


FIGURE 3.

SEASONAL STUDIES OF S. JAPONICUM IN THE SNAIL.



At Shimosanjo the findings for the two years are quite opposite, the infection rate being relatively high at mid year, and relatively low for the same period in 1948.

The Mutsusawa findings are also contradictory. A mid-summer infection peak occurred during 1948, but was completely lacking for 1949. The sharp rise in December of the last year may be valid for the population under consideration, since the infections are new.

LABORATORY INFECTIONS OF SCHISTOSOMIASIS IN THE SNAIL, ONCOMELANIA NOSOPHORA: Shedding tendencies of cercariae of S. japonicum in experimentally infected snails are still being studied. During the summer of 1949, large number of O. nosophora were experimentally infected with the miracidia of S. japonicum. Laboratory raised snails as well as those from the field were infected. Under laboratory conditions the development of these infections has been very slow, but during December 1949, some of the snails were found to contain mature cercariae. To date a series of 9 experimentally infected snails have been isolated and set up daily in glass sputum cups for studies on shedding of cercariae.

STUDIES ON SCHISTOSOME DERMATITIS IN THE JAPANESE: Much dermatitis in Japan is mycotic in origin. However, one type called "kabure" has been attributed to the penetration of hookworm and other larvae into persons working in the fields. The Japanese have recognized for about 30 years a second type called "koganbyo" or "lakeside disease" which occurs in a limited area around Lake Shinji in Shimane Prefecture. However, until recently the etiology of this disease was unknown. Largely through the efforts of Japanese scientists headed by Dr. Hiroshi Tanabe a cercaria of a hitherto undescribed species of a bird schistosome (Gigantobilharzia struniae, n. sp.) was determined to be the etiologic agent of this disease. The disease occurs at a definite place, at a particular season of the year and under a definite combination of circumstances. The endemic area lies on the west side of Lake Shinji and the disease is especially prevalent near the delta of the Hi-i River and the adjacent area along its northwest bank. The disease occurs between April and October but reaches its peak from the latter part of June until early August. This coincides with the period when the paddies are first intensively weeded. Interestingly enough, this is also the period when the greatest numbers of the snail host, Polypylis hemispherula Benson are infected. Table IX summarizes the incidence of infection in the snails during the period when the infection was supposed to be at its height. It appears probable that the snails are introduced from the small feeder irrigation ditches where conditions appear ideal for their propagation at the time the fields are ploughed and flooded. At this time the ridges bearing the grain stubble are worked into the fields as fertilizer. Soon after this the seedlings are planted in the flooded paddies and the stage becomes set for "koganbyo". After the ridges containing the grain have been worked into the soil, many of the snails present become buried in the mud; however, once the snail is freed it comes to the surface and floats. It has been found that agitation of the mud and vegetation caused by walking through a paddy is sufficient to free many snails so that they come to the surface where they can then be collected. In this way snails were collected for use in subsequent experiments.

The proposal of using this dermatitis for clinical evaluation of protective ointments was followed.

PRELIMINARY TESTS WITH PROTECTIVE OINTMENTS: Preliminary tests of methods for protective ointments against Gigantobilharzia struniae were run on a total of 22 persons from the endemic "koganbyo" area near Shutto-mura in Shimane Ken (Table X). Areas about two inches in diameter were painted on the flexor surface of the forearm of each volunteer. Solutions of copper oleate, dibutyl phthallate and dimethyl phthallate were used. The test consisted of placing between 5 to 20 viable cercariae of G. struniae on the painted area and an approximately equal number on a bit of unprotected, normal skin. When a prickling or itching sensation was felt in the control drop penetration was verified by examining the area with a hand lens for evidence of petecchiae. If substantiated it was wiped dry. The drops on the painted areas were left for another 5-15 minutes. All cases in which the untreated, or control area, failed to produce a sensation

were discarded on the assumption that the cercariae were immature and hence incapable of readily penetrating the human skin. None of the persons tested became infected by the cercariae placed on the protected areas of the skin. All of the unprotected, or control areas gave evidence of penetration. The time interval required for penetration varied from 3 to 15 minutes.

Table IX. Summary of Polypylis Specimens Collected

Villages or Burakus	Number of Polypylis Collected	Number Infected	Per cent Infected
Shutto-mura			
Sanbuichi-naka	1,093	90	8.2%
Sanbuichi-kami	341	44	12.9%
Sanbuichi-shimo	182	17	9.3%
Nakanosu-kami	272	26	9.6%
Nakanosu-shimo	1,876	61	3.1%
Okinosu-kami	1,598	52	3.1%
Okinosu-shimo	400	17	4.1%
Kurome-kami	892	62	7.0%
Kurome-shimo	399	17	4.3%
Sakata-naka	217	10	4.6%
Sakata-kami	425	26	6.1%
Sakata-shimo	191	5	2.5%
Meguro-kami	181	15	8.3
Hisagi-mura	6	1	16.6%
TOTALS	8,073	443	5.4%

Table X. Laboratory Tests with Protective Ointments

No. of People Tested	Control Positive In	Minutes Arms Protected by Copper Oleate	Dibutyl-Phthallate	Dimethyl-Phthallate
10	11-20 min.	20	30	
12	9-27 min.	30	30	30

Field Trials of Protective Ointments - The next step consisted of preliminary field trials that were conducted on a small group of 11 persons (Table XI). The ointments were applied to the back of hands and between the fingers. The individuals then worked in the paddies for 1-4 hours. At the end of this period viable cercariae were placed on the treated areas as well as on an untreated control area where it was allowed to stand for some 30-45 minutes. In three cases no evidence of infection occurred in the control but they are included for the sake of completeness.

Specificity of S. japonicum Antigen - It seemed desirable to check the specificity of the Schistosoma japonicum antigen in connection with the existence of "koganbyo" near Shutto-mura in Shimane Ken. A number of volunteers who were known to have suffered with "koganbyo" were injected with 0.01 ml. of a lipoid free fraction of S. japonicum using a 1:10,000 dilution along with saline controls. Five others who had not been exposed served as additional controls. A positive reaction was considered to have occurred if

the wheal showed an increase of 3 mm. ten minutes after injection. In all, a total of 51 persons with "koganbyo" were given a skin test with S. japonicum antigen. Fifteen of these were simultaneously tested with cercariae of G. struniae on the opposite arm. None of these gave a positive nor a delayed reaction in 6, 12, or 24 hours. This is especially interesting in view of the fact that many of the persons who were skin tested were highly sensitive to the dermatitis producing cercariae of G. struniae. Three of these showed marked erythema, both at the site of injection of the antigen and the control, yet the increase in the wheal did not reach 3 mm. It is assumed that the antigen of S. japonicum contained some fraction that is common to the entire schistosome group since antigens from tramatodes other than blood flukes have yielded positive results in skin tests (7, 8, 9, 10). Consequently, it is all the more remarkable that no false positive reactions were obtained. This absence of a response suggests that the antigen used was more specific than it was first believed to be. The potency of the antigen was also checked on known positive cases of schistosomiasis japonica which resulted in positive reactions in 9 out of 10 persons who were passing eggs in the stool.

Table XI. Field Trials with Protective Ointments

Case No.	Control Positive In	Minutes Arms Protected by: Copper Oleate	Dibutyl-Phthallate	Dimethyl-Phthallate
1	10 min.	3½	3½	
2	12 min.	3½	3½	
3	20 min.	3½	3½	
4	17 min.	4	4	
5	37 min.	4		
6	21 min.	3 3/4	3 3/4	
7	10 min.	2 3/4		
8	-	2½	2½	
9	16 min.		2½	2½
10	-	1		1
11	-	1		1

Cross Infection Experiments with Bird and Human Schistosomes - In these experiments an attempt was made to determine whether or not animals develop a sensitivity to repeated infections by: (a) the cercariae of the bird schistosome, G. struniae, (b) the cercariae of the bird schistosome followed by cercariae of S. japonicum, (c) the repeated exposure to cercariae of S. japonicum and (d) the repeated exposure to cercariae of S. japonicum followed by those of the bird schistosome. It was hoped that these experiments would throw some light on the sensitivity produced in man by repeated exposure to the bird schistosomes.

In attempting to determine sensitivity, both mice and rabbits were utilized. The initial exposures were made using varying numbers of cercariae depending upon the host. One to three exposures were made within a 10 day period on the sensitizing exposure to the same or other species approximately 3 weeks later.

The technique of exposure was varied of necessity because of the different tropic responses of the cercariae being used. In the case of S. japonicum it is well recognized that the cercariae tend to collect on the surface film. In the case of mice the back of the neck, and in rabbits the abdomen was shaved. The animals were rested and then placed with the exposed surface in contact with the surface film. Since the cercariae of G. struniae do not react in this manner a known number of cercariae was placed within a glass or bamboo ring on the shaven area for 10-30 minutes, or 2-3 crushed infected snails were placed on the area for the same interval, after estimating the number of viable cercariae present. Each exposed area was carefully searched for macroscopic changes including petechiae, macules, or edema. Biopsies were made at intervals of 5 and 30 minutes and 1, 2, 5, 12, 24 and 48 hours and these were preserved, sectioned and examined.

Effects of Repeated Exposure to Cercariae of Bird Schistosomes - The initial exposure of 11 rabbits to 50-600 cercariae each of the bird schistosome, G. struniae, resulted in evidence of reactions, such as petecchiae, macules or edema in only one case. A second exposure two weeks later of these rabbits yielded four reactions. A third exposure 4-5 weeks subsequently yielded reactions in 7 of the 11 rabbits. This indicates that some of the rabbits react to repeated doses of cercariae of the bird schistosomes. Even though there was microscopic evidence of reaction in 7, all but one of the 11 animals showed reactions when the sections of the biopsies taken after 5 and 30 minutes, 1, 5 and 24 hours after exposure were studied. Cercariae were identified in all cases and evidence of a clear cut host reaction was seen. There was cellular infiltration in the tissue and evidence of congestion of the contiguous capillaries with minute hemorrhages into the surrounding tissue. After re-exposure a month later, biopsies were repeated. There was clear cut evidence of an increased reaction (Table XII). This is suggestive of the clinical picture in man where sensitization occurs in many cases following repeated exposures to non-human schistosomes (11, 12, 13, 14, 15, 16).

Table XII. Histological Findings in Rabbits After Exposure to Bird Schistosomes

Biopsy Made In	Cercariae Present	CELLULAR REACTION Around Cercariae	CELLULAR REACTION In Tissue	Hemorrhage in Tissue	Congestion of Capillaries	Edema of Tissue
Initial Exposure						
30 min.	+	-	±	+	+	-
1 hr.	+	-	±	2+	+	-
5 hrs.	+	±	±	+	2+	-
24 hrs.	+	+	+	2+	2+	±
Second Exposure to Bird Schistosomes One Month Later						
30 min.	+	+	+	±	+	-
1 hr.	+	2+	2+	+	2+	±
5 hrs.	+	3+	3+	+	3+	3+
24 hrs.	+	4+	4+	+	3+	3+

Key: - No reaction
± Slight
\+ Moderate
2+ Considerable reaction noted
3+ Marked reaction noted
4+ Very marked reaction noted.

Mice in general, appear less reactive than rabbits. In a series of 8 there was no detectable reaction after the first exposure and only one animal showed anything after the second. Repeated dosages are contemplated to determine whether or not mice may be sensitized.

Effects of Exposure to Bird Schistosomes Followed by Cercariae of S. japonicum - Five of the rabbits which had been exposed to cercariae of the bird schistosome on three occasions (vide supra) were utilized. Four to five weeks after the last exposure these animals were exposed to 200 cercariae each of S. japonicum. There was no evidence of increased sensitivity either dermatologically or in the sectioned tissue (Table XIII). In fact parasites were not recovered in the tissues except in two cases.

In a small series of only three mice which were exposed first to cercariae of G. struniae there was no visible or histologic evidence of reaction a month later when exposed to 100 cercariae each of S. japonicum. These were subsequently autopsied. There was no evidence of any immunity as approximately the same numbers of adult worms were recovered from both the experimental animals, and from controls which had been exposed only to S. japonicum.

Effects of Repeated Exposure to Cercariae of S. japonicum - Each of six rabbits was exposed to 300-500 cercariae of S. japonicum. At the end of 2 and 6 weeks the exposure was repeated. Biopsies seldom revealed cercariae and there was virtually no evidence of any pathologic or immune reaction. In the absence of any immune reaction these findings are not considered as unusual for rabbits serve as a good host for S. japonicum.

Table XIII. Histological Findings in Rabbits After Initial Exposure To Bird Schistosomes Followed by S. japonicum.

Initial Exposure to Bird Schistosomes

Biopsy Made In	Cercariae Present	Cellular Reaction Around Cercariae	Cellular Reaction In Tissue	Hemorrhage In Tissue	Congestion of Capillaries	Edema of Tissue
30 min.	+	-	±	+	+	-
1 hr.	+	-	±	2+	+	-
5 hrs.	+	±	+	+	2+	-
24 hrs.	+	+	+	2+	2+	±

Second Exposure to S. japonicum one Month Later

No reaction or parasites detected in 30 min., 5 hrs., or 24 hours

Key: See Table XII

Each of a total of 36 mice were exposed to 25-200 cercariae of S. japonicum. Two weeks later the dose was repeated. There was no detectable dermatologic or histologic reaction seen. In only one case were cercariae found after five hours. Such findings are probably not unusual since mice serve as normal hosts for S. japonicum and the cercariae penetrate rapidly.

Exposure to Cercariae of S. japonicum followed by the Bird Schistosome - To date only six rabbits were initially exposed to 200-600 cercariae of S. japonicum. Nearly six weeks later one animal was exposed to approximately 100 cercariae of G. struniae and the others to 350 or more. There was no detectable dermatologic reaction in either the initial or second exposure. However, sections revealed cercariae present as well as some cellular infiltration (Table XIV). The reaction appeared to be less intense than in the case where G. struniae had been used initially. This is suggestive but requires further work which must perforce await the dermatitis season of 1950.

EPIDEMIOLOGICAL STUDIES IN THE FAR EAST - During 1949 the long term program of epidemiologic studies was extended to Shikoku, Hokkaido, and Okinawa. During the survey of Okinawa a total of 340 Americans were examined. These individuals were primarily from two units and represented both white and colored personnel. Of the 340 persons examined (Table XV) 34.3 percent were infected, 4.4 percent with helminths and 31.6 percent with protozoa. This group was divided into those who had been there less than three months and those who had been on Okinawa longer. Although the numbers are small it appears that there was an increase of approximately 12 per cent in those who had been in the island for the longer period. This compares with about a 4 per cent increase in Japan and an 8 per cent increase in South Korea, previously reported (17).

Table XIV. Histological Findings in Several Rabbits After Initial Exposure To *S. japonicum* Followed by Bird Schistosomes

Initial Reaction in Rabbits and Mice

Biopsy Made In	Cercariae Present	Cellular Reaction Around Cercariae	Cellular Reaction In Tissue	Hemorrhage In Tissue	Congestion of Capillaries	Edema of Tissue
No reaction or parasites detected in 30 min., 1 hr., 5 hrs., or 24 hrs., in either rabbits or mice						
Reactions in Rabbits After 2nd Exposure to Bird Schistosomes Six Weeks Later						
30 min.	+	-	-	-	±	-
1 hr.	+	-	-	-	±	-
5 hrs.	+	±	+	-	+	-
24 hrs.	+	+	+	-	-	-
Reactions in Mice after 2nd Exposure to Bird Schistosomes Six Weeks Later						
30 min.	+	-	-	-	-	-
1 hr.	+	-	-	-	-	-
5 hrs.	+	-	-	-	-	-
24 hrs.	+	±	+	-	±	-

Key: See Table XII

Table XV. Comparison of the Incidence of Intestinal Parasites in American Personnel in Japan, Korea and Okinawa

	Americans in Japan				Americans in Korea				Americans in Okinawa			
	1 to 3 months		Over 3 months		1 to 3 months		Over 3 months		1 to 3 months		Over months	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. examined	174		1534		86		241		26		341	
No. parasitized	58	33.3	571	37.2	19	22.1	73	30.3	6	23.1	111	35.4
No. with helminths	9	5.2	86	5.6	1	1.2	20	8.3	0	0.0	15	4.8
No. with protozoa	51	29.3	527	34.4	18	20.9	62	25.7	6	23.1	102	32.5
Helminths:												
Ascaris	1	0.6	22	1.4	0	0.0	5	2.1	0	0.0	2	0.6
Whipworm	4	2.3	26	1.7	0	0.0	3	1.2	0	0.0	6	1.9
Hookworm	5	2.9	38	2.5	1	1.2	11	4.6	0	0.0	8	2.5
Strongyloides stercoralis	0	0.0	0	0.0	0	0.0	1	0.4	0	0.0	2	0.6
Hymenolepis nana	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0
Metagonimus sp.	0	0.0	0	0.0	0	0.0	1	0.4	0	0.0	0	0.0
Protozoa:												
Endamoeba histolytica	9	5.2	91	5.9	3	3.5	12	5.0	1	3.8	16	5.1
E. coli	29	16.7	259	16.9	11	12.8	34	14.2	3	11.5	48	15.3
Endolimax nana	20	11.5	256	16.7	6	7.0	25	10.4	5	19.2	50	15.9
Iodamoeba butschlii	1	0.6	6	0.4	0	0.0	1	0.4	0	0.0	6	1.9
Giardia lamblia	7	4.0	95	6.2	3	3.5	9	3.7	0	0.0	16	5.1
Chilomastix mesnili	0	0.0	1	0.1	1	1.2	0	0.0	0	0.0	0	0.0

SURVEY FOR INTESTINAL AND BLOOD PARASITES IN THE JAPANESE: Introduction - A series of extensive epidemiological surveys in Japan have been carried out beginning in 1947 as a cooperative project with the Japanese National Institute of Health. Although the work accomplished in 1947 and 1948 is not included in this report, some of the areas surveyed will be mentioned and some of the data included in the tables for the sake of comparison and completeness. The surveys contemplated for Shikoku and Hokkaido have been completed. Numazu remains with perhaps one or two additional areas such as Sèndai and Niigata.

Methods - These are reiterated here to allow for continuity. To accomplish the desired objectives an epidemiologic data sheet is completed on each person examined, the questioning being done by Japanese physicians. The same Japanese physicians perform a brief examination on each person, which includes checking for evidence of hepatomegaly, splenomegaly, anemia, jaundice, ascites, and cheilosis, and lymphadenopathy. The general state of health is recorded. Finally in some instances, blood smears for malaria or filariasis, as well as perianal swabs for pinworms are made.

A minimum of 1200 persons and a maximum running to over 3000 have been examined in each geographical area where a survey has been carried out. Every effort is made to secure representative samplings of both sexes as well as all age groups and economic strata. In many instances it has been necessary to limit the study group to a single occupation.

Following the physical examination, the stool specimen is examined by the MGL (18) and AMS III (19) techniques for ova and protozoa. (Both of these techniques are modifications of the original Telemann acid-ether centrifugation method). Finally, direct smears are examined to obtain figures that have some comparative value with earlier surveys, and an arbitrary rough calculation of the density of parasitism made. Throughout the surveys an attempt to maintain a constant base line has been made by using the same techniques, the same supervisors, the same technicians, and the same Japanese physicians.

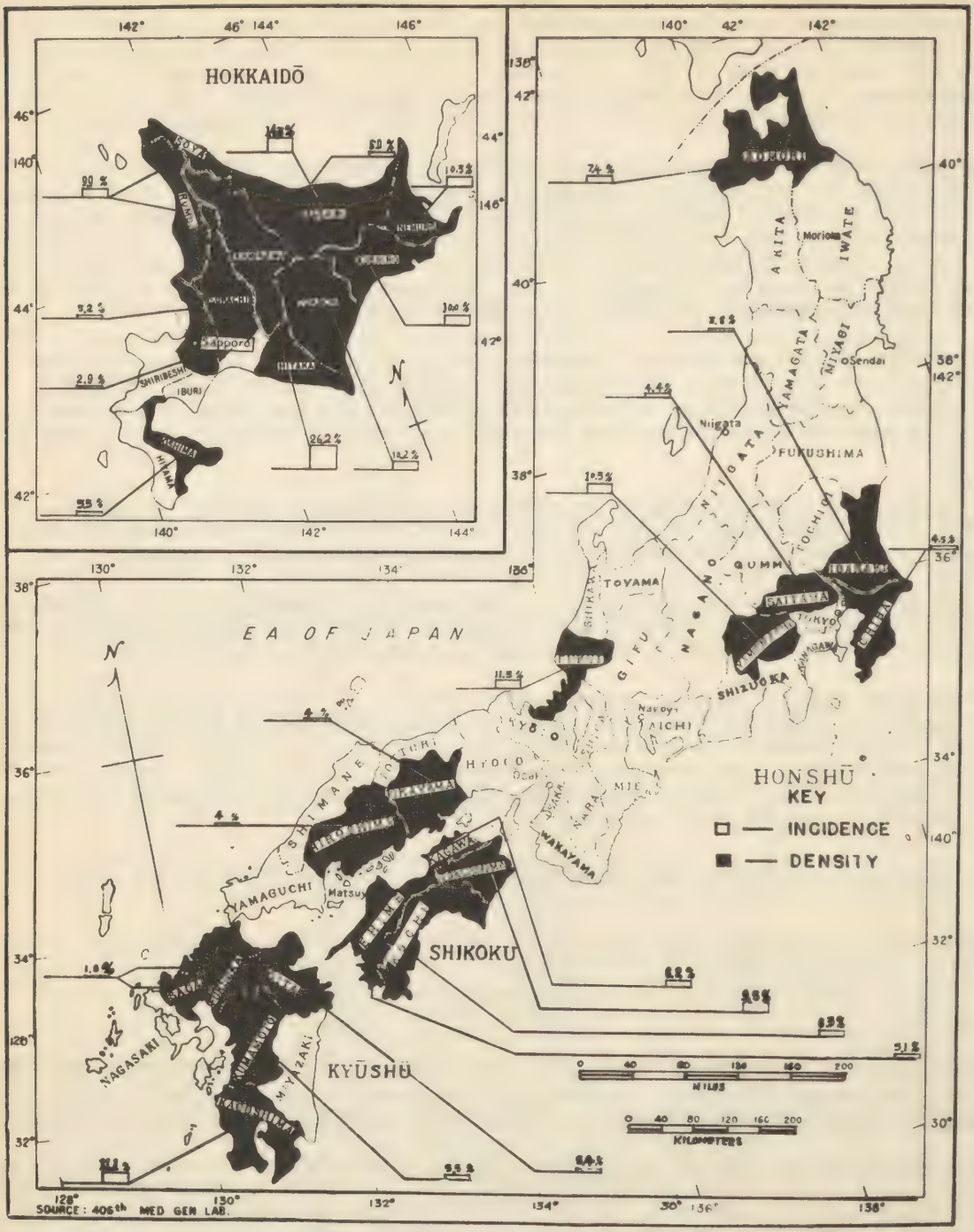
A parasite density index or factor has been used in an attempt to secure information on the degree or intensity of the infection, something which is not usually tried on more than one or two species of parasites. While the index is an arbitrary figure, nevertheless the results can be compared and these show clearly how the picture varies from one region to another. The index is reached as follows: the number of eggs (or cysts) in each microscopic preparation is recorded

Symbol	Actual Count	Index Figure
1	1-9	5
1+	10-49	30
2+	50-99	75
3+	100-199	150
4+	200-399	300
5+	400+	500

The total number of cases in each category is multiplied by the index figure. These figures are totalled and divided by the number of infected persons. The resulting figure is the parasite density index or factor. In this way a rough but usable index is obtained.

Present Status of Analysis of Results - In the discussion that follows only the more important parasites have been selected and tabulated. It has been impossible even yet to fully analyze all of the data. In the overall picture the incidence of parasitism is extremely high, with 90 to 95 percent of the individuals examined being infected with one or more helminths, while somewhat less than half were infected with one or more protozoa. The protozoan rates serve as a better index of contamination of food or water than the worms which are more nearly universally present. However, the incidence of protozoa taken together with the density index of the parasitic worms is sufficient to provide a quick picture of a given area since both are higher when sanitary conditions are poorer. A summary of the incidence and density by species of parasite is presented in Figures 5, 6 7, 8, 9, 10, 11. One of the most striking situations occurs in Kofu in Yamanashi Prefecture where the incidence of both worms and protozoa is high. There whipworm is more prevalent than ascaris which is usually regarded as the most widely distributed worm in Japan. In general the incidence and density of the parasitism is probably due to a combination of interesting factors which include: the length of time that the night soil was stored,

FIGURE 5

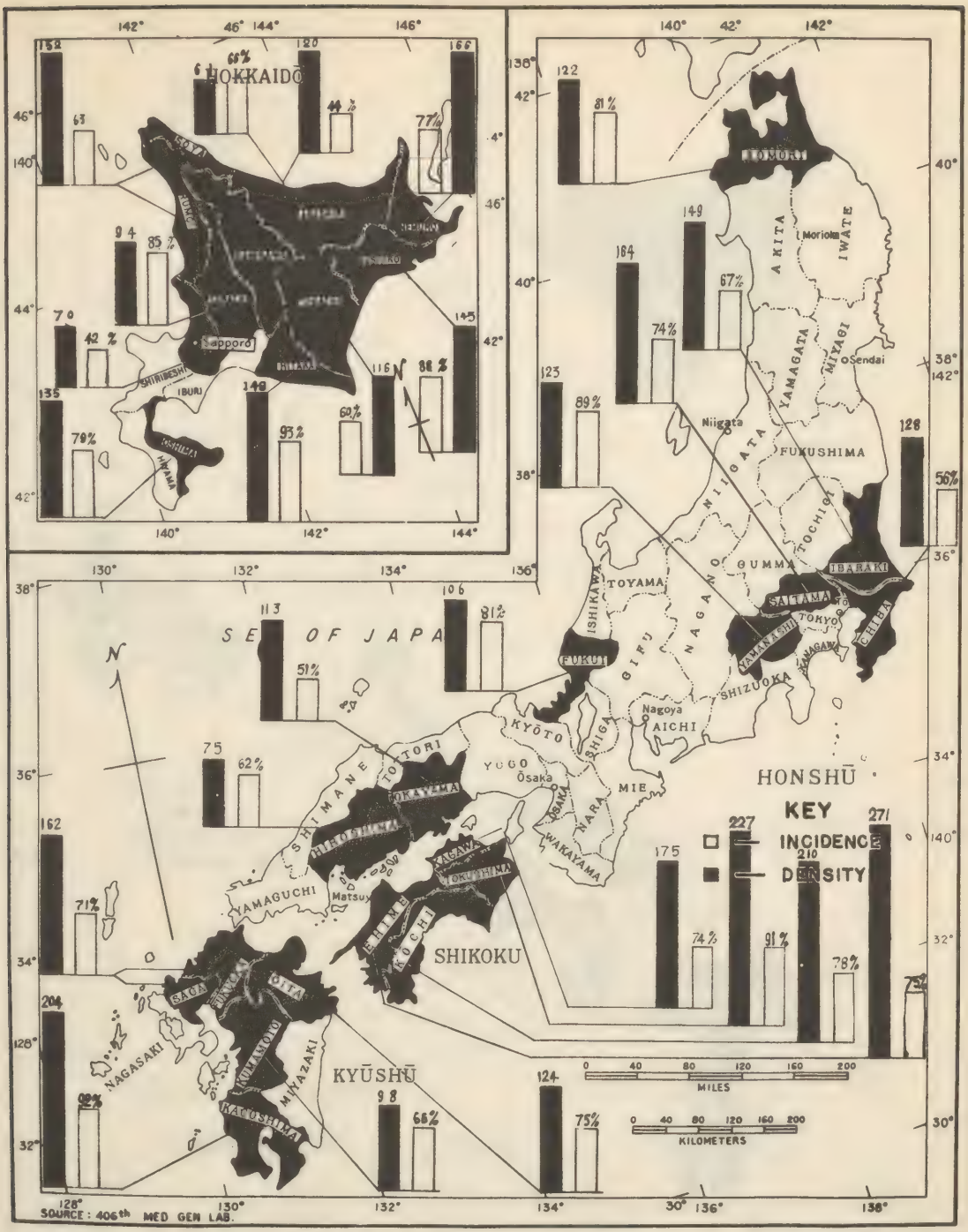


AMEBIASIS — INCIDENCE

5-10-20-8-47-64 ENGR-500

Sect. Med. Zool.
406 Med. Gen. Lab.

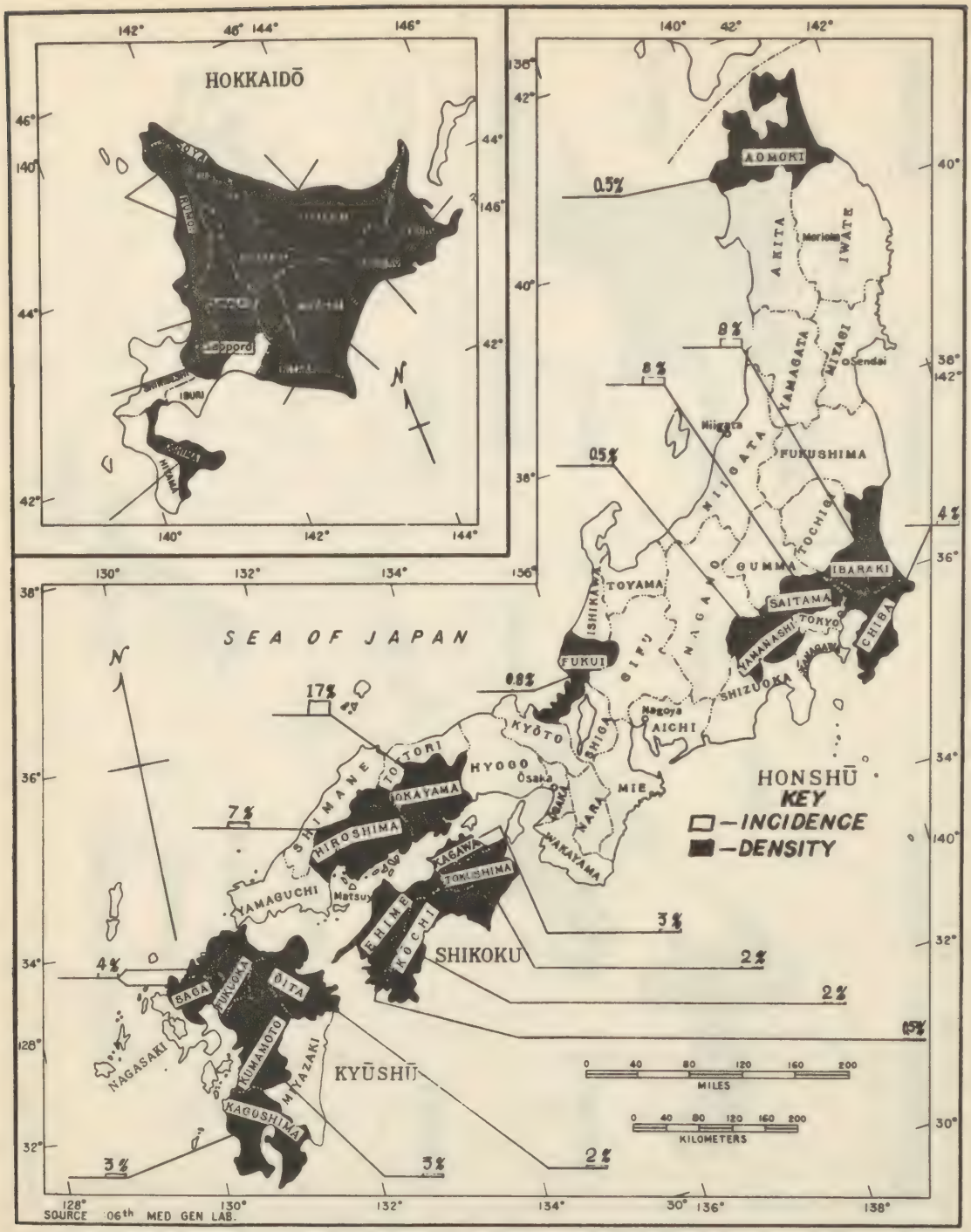
FIGURE 6



ASCARIS — INCIDENCE AND DENSITY

5910-20-8-47-64ENGR-500
Sect. Med. Zool.
406th M.G. Lab.

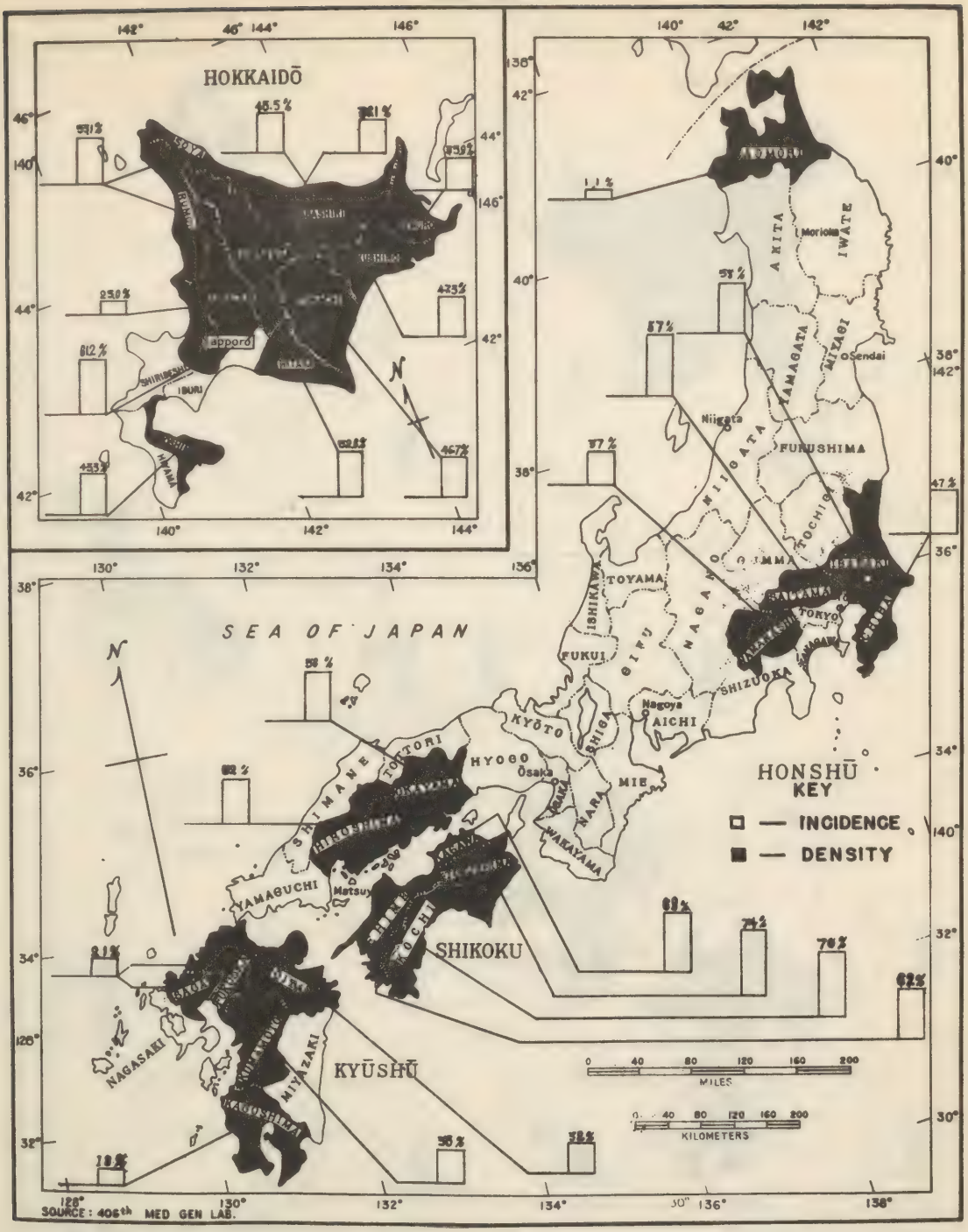
FIGURE 7



CLONORCHIASIS—INCIDENCE AND DENSITY

5910-20-8-47-64 ENGR-500
Sect. Med. Zool.
406TH M.G. Lab.

FIGURE 8

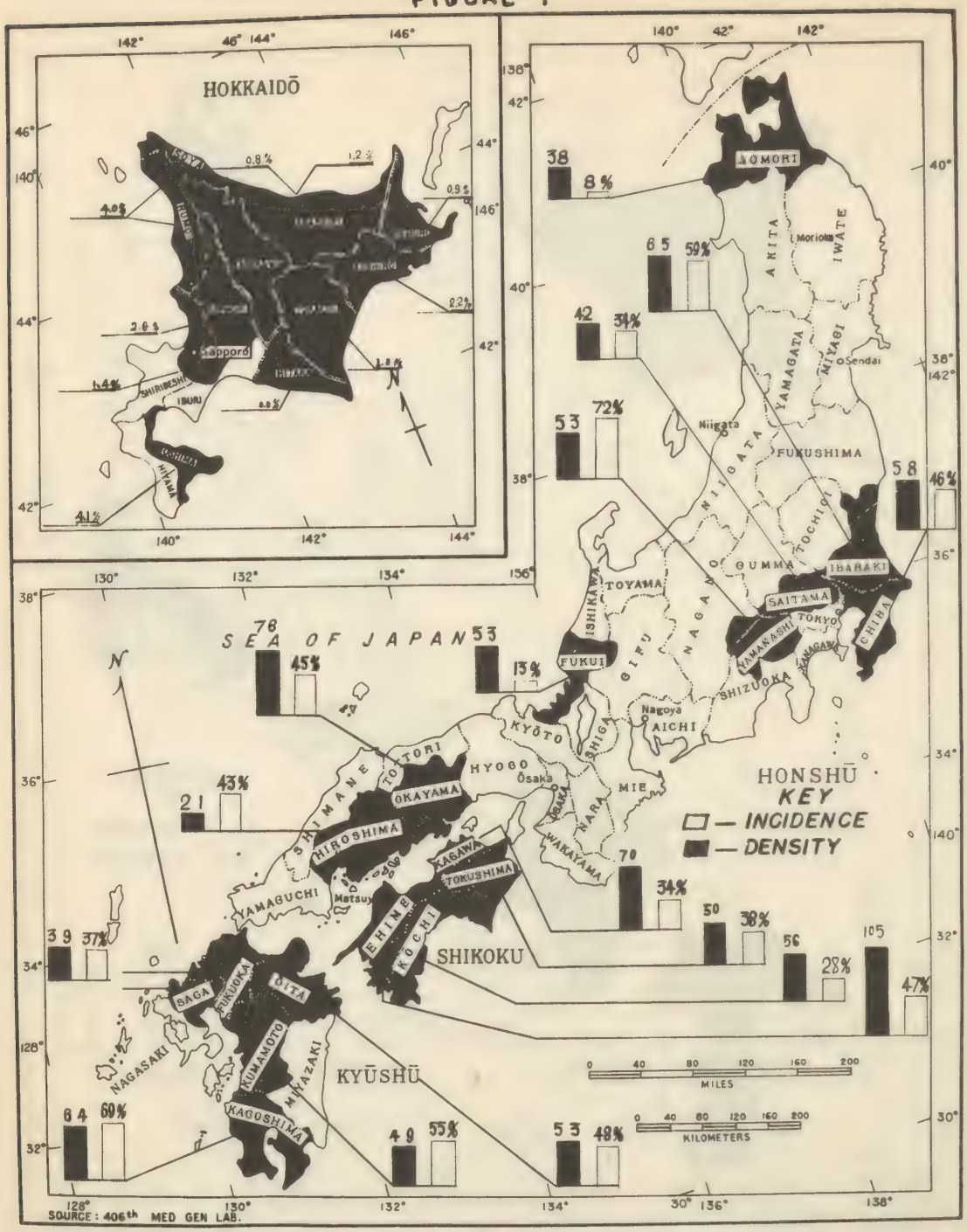


ENTEROBIASIS — INCIDENCE

5910-20-8-47-64ENGR-300

Sect. Med. Zool.
406 Med. Gen. Lab.

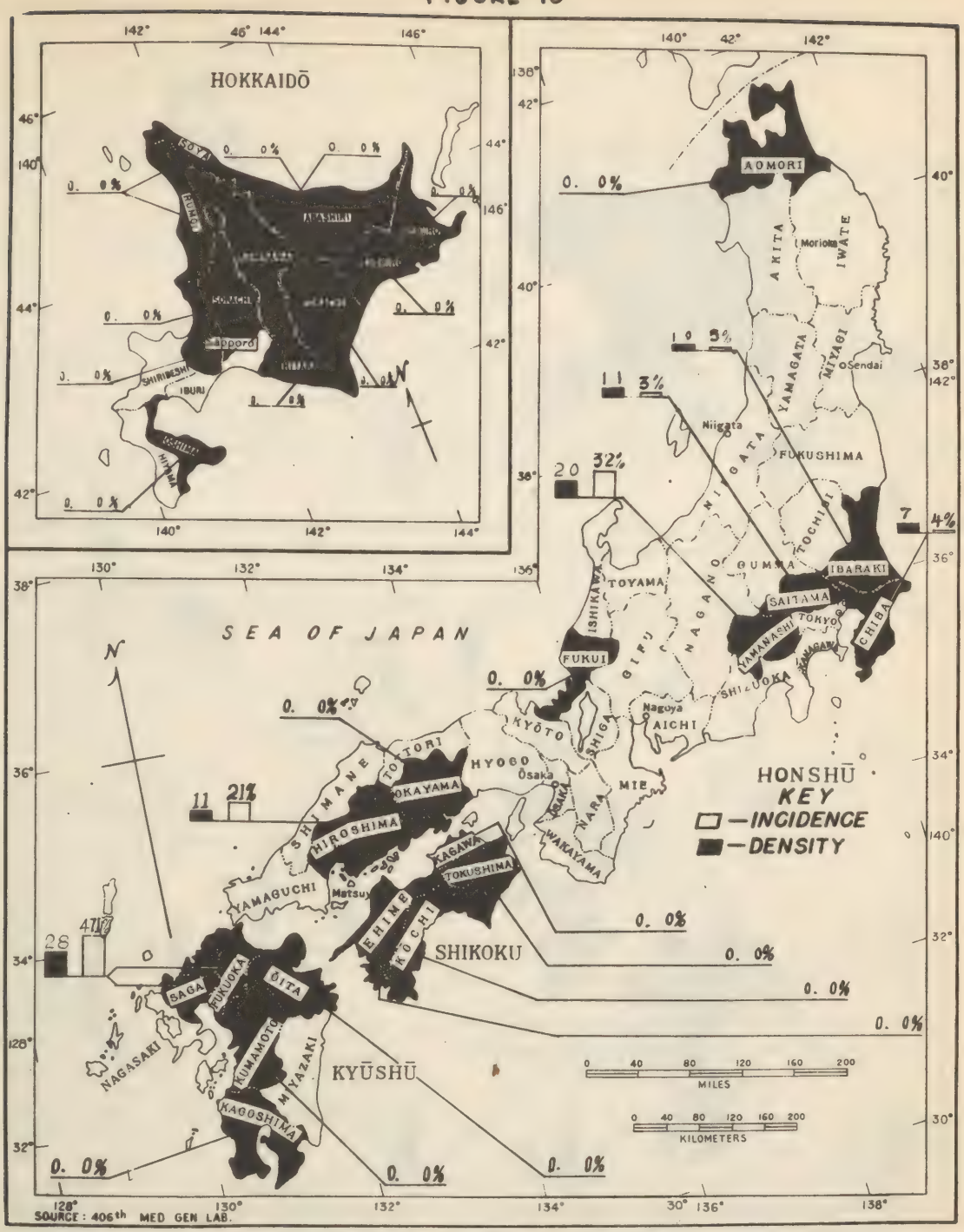
FIGURE 9



HOOKWORM-INCIDENCE AND DENSITY

5810-20-8-47-64 ENGR-500
Sect. Med. Zool.
406TH M.G. Lab.

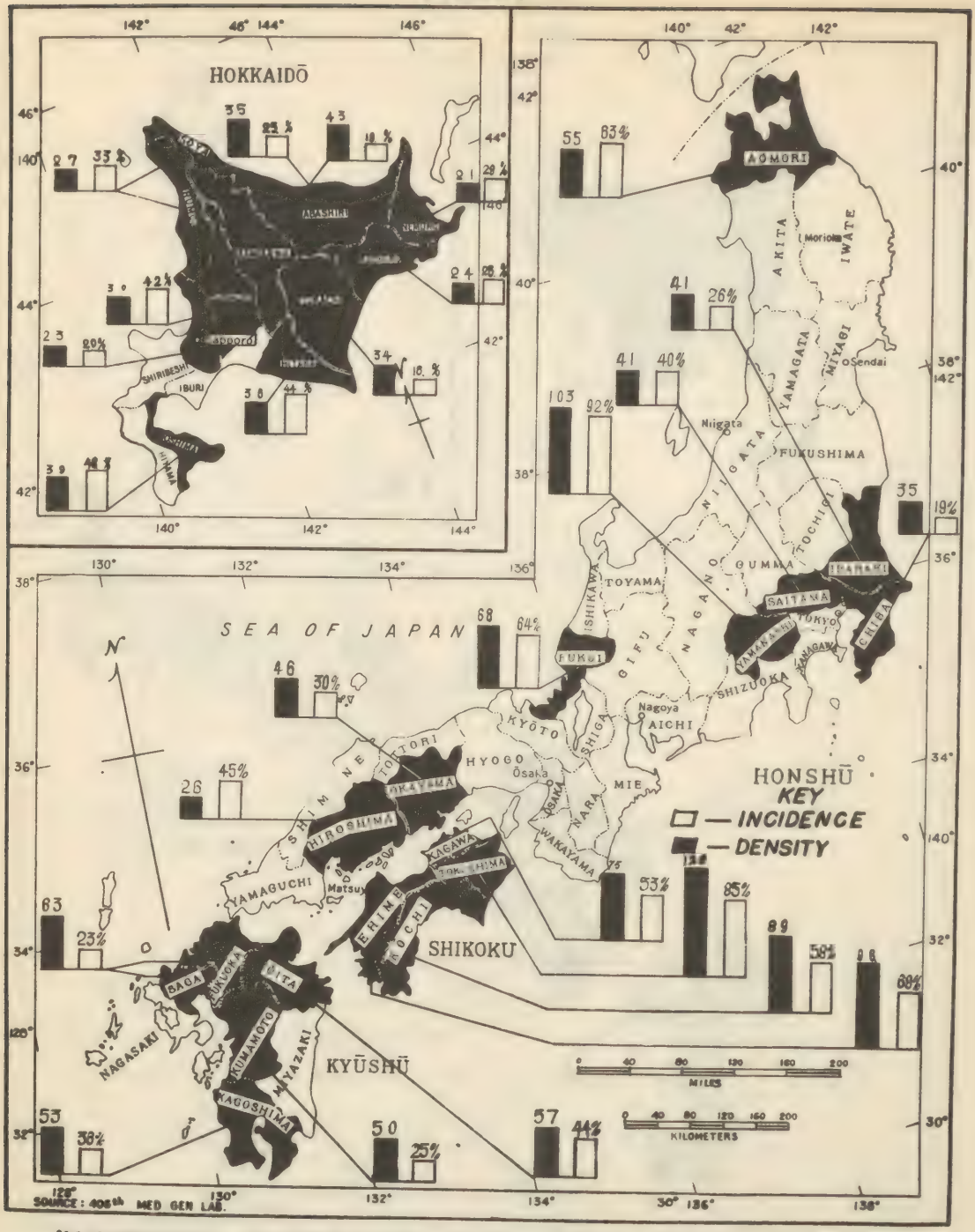
FIGURE 10



SCHISTOSOMIASIS—INCIDENCE AND DENSITY

5910-20 8-47-64ENGR-500
Sect. Med. Zool.
406TH M.G. Lab.

FIGURE 11



WHIPWORM—INCIDENCE AND DENSITY

3910-20-8-47-64ENGR-200
Sect Med Zool.
406TH M.G.Lab.

location of the population group studied (for some worms such as ascaris appear to be more prevalent in the hillside communities), the presence of door-yard gardens and whether or not they are fertilized by fresh night soil, the frequency with which fields, wells or houses are flooded, the type of farming, and others. These various factors are in the process of being evaluated at the present time.

EPIDEMIOLOGIC SURVEY OF SHIKOKU: The survey logically falls into four areas of study which are summarized in Table XVI and Figure 12. It will be seen from this, that as in the past, attempts were made to examine population groups from both the hillsides and the low flat areas. As indicated, 1720 individuals were examined for intestinal parasites. Of the above group, 83 submitted sputum specimens for the detection of paragonimiasis. Blood specimens were collected from an additional number, 369 for malaria and filariasis.

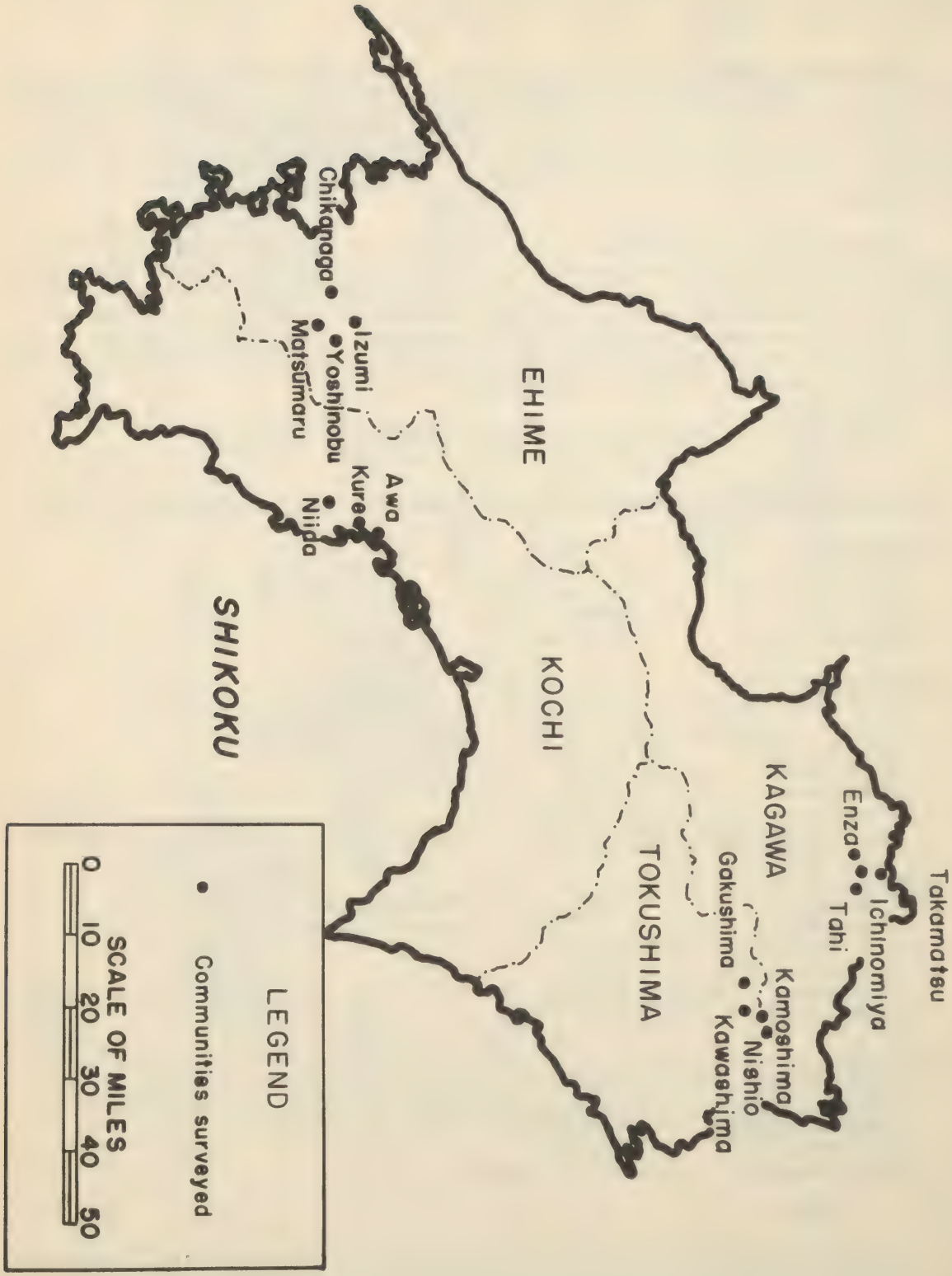
It is interesting to note that fresh, unstored night soil appears to be used with greater abandon on the door yard gardens and fields on Shikoku than in many other areas of Japan that have been surveyed. It was the exception rather than the rule to find that night soil was stored at all before use. Furthermore in some areas of Shikoku where water is more scarce the bath and other waters are often added to the night soil, thus diluting it and probably increasing the survival possibilities of eggs which otherwise might be destroyed by more highly concentrated night soil.

Table XVI. Summary of Areas and Communities Studied on the Shikoku Survey - April - May, 1949

Area and Community	Character of Area	Reason for Survey	No. Examined	Total of Area
Kagawa-Ken				
Tahi-mura	Farming	Routine	113	
Ichinomiya-mura	Farming	Routine	118	
Inza-mura	Farming	Routine	118	
Takamatsu-shi	Urban	Routine	101	450
Tokushima-Ken				
Yamada-buraku	Farming	Metagonimiasis and heterophyliasis	117	
Gaskushima-mura (Tsuji-buraku)	Farming	"	113	
Nishiio-mura (Ino-buraku)	Farming	"	109	
Kamojima-mura	Urban	"	113	442
Kochi-Ken				
Suzaki-machi (Awa-buraku)	Farming	Coastal area, filariasis and malaria	100	
Tosa-Kure-machi	Farming	"	111	
Tosa-Kure-machi	Fishing	"	101	
Niida-mura (Niida-buraku)	Farming	"	103	415
Ehime-Ken				
Yoshinobu-mura (Yoshino-buraku)	Farming	Paragonimiasis	104	
Chikanaga-machi	Farming	"	104	
Izume-mura	Farming	"	102	
Matsumaru-machi	Urban and Farming	General	103	413
TOTAL				1,720

Attempts were made to secure data on the presence of schistosomiasis. The stools from one family in Kochi-Ken, who was reported to have this disease, were carefully examined by means of the AMS III technique; only ascaris and whipworm ova were found. No evidence of any indigenous cases of schistosomiasis could be found elsewhere. It appears that this disease is not endemic on the Island of Shikoku.

Figure 12


Takamatsu
Ichinomiya
Enza
Tahi
KAGAWA
Kamoshima
Nishio
Gakushima
Kawashima
TOKUSHIMA
KOCHI
EHIME
SHIKOKU
Chikanaga
Izumi
Yoshinobu
Matsumaru
Awa
Kure
Niida
LEGEND
Communities surveyed
SCALE OF MILES
0 10 20 30 40 50

Epidemiological Findings in Kagawa Ken - The overall incidence of intestinal parasitism for this area (Table XVII) averaged 94.5 per cent with surprisingly little variation between the communities which were examined. The incidence of protozoa, 48.2 per cent, was sufficiently high to suggest extraordinary contamination of the water and possible food. The incidence of Endamoeba histolytica was not unduly high although the variations found must be interpreted as being due to the difference in the sanitary conditions in the respective communities. It will also be seen from Table XVII that the incidence for the various helminths is considerably less than was found in many other surveys. While the density has not yet been computed it is believed the lower incidence may be due in part to the drier climate as there is reported to be less rain than in other areas. This would tend to cause the destruction of the less resistant parasite eggs such as hookworm and whipworm.

Table XVII. Summary of the Incidence of Intestinal Parasites in Kagawa-Ken

Character of Community	TAHI-MURA Farming		ICHINOMIYA-MURA Farming		ENZA-MURA Farming		TAKAMATSU-CITY Urban		TOTAL	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. Examined	113		118		118		101		450	
No. Parasitized	106	93.8	111	94.0	110	93.2	98	97.0	425	94.5
No. with Protozoa	47	41.5	58	49.1	53	44.9	59	58.4	217	48.2
No. with Helminths	104	92.0	106	89.8	109	92.3	95	94.0	414	92.0
No. with										
E. histolytica	1	0.8	12	10.1	9	7.6	7	6.9	29	6.4
E. coli	36	31.8	36	30.5	36	30.5	46	45.5	154	34.2
Endolimax nana	22	19.4	26	22.0	27	22.8	27	26.7	102	22.6
Giardia lamblia	2	1.7	7	5.9	4	3.3	4	4.0	17	3.8
No. with										
Ascaris	83	73.4	89	75.4	87	73.7	73	72.2	332	73.8
Whipworm	55	48.6	54	45.7	72	61.0	55	54.5	236	52.5
Hookworm	33	29.2	54	45.7	43	36.4	24	23.8	154	34.2
Trichostrongylus sp.	5	4.4	7	5.9	3	2.5	6	5.9	21	4.7
Clonorchis sinensis	4	3.5	3	2.5	6	5.0	1	-	13	2.9
Heterophyes sp.	-	-	-	-	-	-	2	2.0	2	0.4
Metagonimus sp.	4	3.5	1	0.8	2	1.7	1	1.0	8	1.8

Epidemiological Findings in Tokushima-Ken - The valley of the Yoshino-gawa was selected as the area to be surveyed in Tokushima Prefecture because it was reported to be typical of the better farming and smaller urban communities of the Ken. By so doing it was not possible to examine persons from the coastal fishing communities; however this was covered by the Kochi-Ken survey. The overall incidence of parasitism averages 98.5 percent with 30.7 per cent carrying protozoa and 98.5 per cent helminths (Table XVIII). In Yamada-buraku it was rather striking to find 10 and 14.5 per cent carrying E. histolytica and E. coli respectively. This ratio is suggestive of an unusual condition such as is found during an epidemic. However, no information was forthcoming on any recent outbreaks of diarrhea. It is possible that this is a normal situation since some of the drinking water sources consisted of open springs or wells that could easily be contaminated by surface drainage from the fields situated further up the hills; Ino-buraku which is a part of Nishio-mura also had an unusual incidence of E. histolytica and E. coli with 14 and 17 per cent respectively. No satisfactory explanation of this situation could be found except the almost universal use of fresh night soil on both the dooryard gardens and the fields. As noted earlier this was often diluted with bath water as well as kitchen wastes, a custom that tends to reduce the possibilities of generating heat and so destroying stored eggs. The two other communities showed less E. histolytica and proportionately more E. coli.

Table XVIII. Summary of the Incidence of Intestinal Parasites in Tokushima-Ken

Character of Community	KAMASHIMA-MACHI Yamada-buraku Farming No.	%	GAKUSHIMA-MURA Tsuji-buraku Farming No.	%	NISHIO-MURA Ino-buraku Farming No.	%	KAMOJIMA-MURA Urban No.	%	TOTAL No.	%
No. examined	117		113		109		113		452	
No. parasitized	117	100	113	100	108	99.9	107	94.6	445	98.5
No. with protozoa	37	31.6	38	33.6	32	29.4	32	29.3	139	30.7
No. with helminths	117	100	113	100	108	99.9	107	94.6	445	98.5
No. with										
Endamoeba histolytica	12	10.2	9	7.9	15	13.8	8	7.0	44	9.7
E. coli	17	14.5	25	22.1	18	16.6	23	20.3	83	18.4
Endolimax nana	19	16.2	16	14.1	13	11.9	7	6.1	55	12.2
Giardia lamblia	3	2.5	4	3.5	1	0.9	2	1.7	10	2.2
No. with										
Ascaris	115	98.2	107	94.6	98	90.0	88	77.8	408	90.2
Whipworm	110	94.0	105	92.0	89	81.8	78	69.0	382	84.6
Hookworm	65	55.5	50	44.2	39	35.8	18	15.9	172	38.2
Trichostrongylus sp.	6	5.1	1	0.8	1	0.9	3	2.6	11	2.4
Clonorchis sinensis	-	-	5	4.4	1	0.9	1	0.8	7	1.5
Heterophyes sp.	7	5.9	8	7.0	4	3.7	12	10.6	31	6.8
Metagonimus sp.	6	5.1	14	12.3	6	5.5	9	7.9	35	7.7

Table XVIII shows that there are slightly fewer intestinal worms in Kamojima-mura. Since the people here are primarily urban, the effect of the absence of daily gardening becomes apparent in the great reduction of hookworm, 36-56 percent in the farming communities compared to 16 percent in kamojima. Kamojima also shows less ascariasis and trichuriasis. This is perhaps due in part to the fact that some vegetables such as dicons which are often prepared locally by the farmers can still carry viable ascaris and whipworm eggs. It is believed that these differences in the incidence of parasitism as noted are real rather than merely apparent.

This was one of the few areas encountered so far where there was an appreciable amount of heterophyiasis and metagonimiasis. Both of these diseases are caused by the ingestion of improperly cooked or frankly raw river or brackish water fish. It will be noted that there was 6.8 and 7.7 per cent respectively of these diseases. It is also significant to note the relative absence of clonorchiasis, a disease which is also carried by many species of fresh-water fishes. Our observations on this led to the conclusion that it was not found largely because of the absence of the proper snail host. Another factor in the higher incidence of these two fish-carried parasites lies in the dearth of animal protein in the diet.

Epidemiological Findings in Kochi-Ken - It was felt desirable to examine people from communities along the southern coast where the climate is somewhat warmer. The overall incidence for the persons studied in these communities near Tosa-Kure was 93.4 per cent parasitized, 40 per cent with protozoa and 91 per cent with helminths (Table XIX). There seemed to be a little more infection with most of the protozoa than in Kagawa-Ken. On the average the intestinal protozoa appeared to fall within the expected limits. In general the parasitic worms had a lower incidence in most communities than in those observed in Tokushima. Ascaris averaged only 78 per cent in Kochi compared with 90 per cent in Tokushima.

One interesting comparison was possible in Tosa-Kure-machi. This town was divided into a fishing community and a farming community. Although the differences are apparent in Table XXII it may be worth while to emphasize them here. The incidence of infection in the fishing community was lower than that in the farming community by over 6 per cent. However, the incidence of protozoa was higher, 59.4 per cent compared with 36, while that of the helminths was lower. As suggested previously (*vido supra*) this

can be accounted for by less exposure to the infective eggs and larvae of the parasites. The increase in protozoa appears to be correlated with the more noticeable lack of cleanliness and general sanitation as well as the prevalence of flies.

Table XIX. Summary of the Incidence of Intestinal Parasites in Kochi-Ken

Character of Community										
No. examined	100		111		101		103		415	
No. parasitized	90	90.0	107	96.4	90	89.0	100	97.2	387	93.4
No. with protozoa	37	37.0	40	36.1	60	59.4	28	27.2	165	39.6
No. with helminths	85	85.0	105	94.6	87	86.1	99	96.2	376	91.0
No. with										
E. histolytica	7	7.0	5	4.5	8	7.9	6	5.8	26	6.3
E. coli	20	20.0	34	30.6	48	47.5	21	20.4	123	29.6
Endolimax nana	13	13.0	20	18.1	23	22.8	14	13.6	70	16.8
Giardia lamblia	10	10.0	3	2.7	5	4.9	2	1.9	20	4.8
No. with										
Ascaris	82	82.0	88	79.3	75	74.2	79	76.7	324	78.0
Whipworm	39	39.0	74	66.7	61	60.4	67	65.0	241	58.0
Hookworm	20	20.0	32	28.8	16	15.8	45	43.6	113	27.3
Trichostrongylus sp.	3	3.0	22	19.8	4	3.9	1	1.0	30	7.2
Clonorchis sinensis	1	1.0	-	-	3	2.9	5	4.9	9	2.2
Heterophyes sp.	-	-	3	2.7	-	-	8	7.8	11	2.7
Metagonimus sp.	3	3.0	4	3.6	3	2.9	21	20.4	31	7.5

The public health centers reported the presence of a certain amount of filariasis and malaria. This was given some credence since more evidence of lymphadenopathy was noted and there appeared to be more splenomegaly. Blood was examined for microfilariae and thick and thin smears were made for the detection of malaria. A total of 367 persons were examined by thick smears for malaria. None was found. Blood samples were taken from 369 persons for examination for microfiliariae. Only a single microfilaria was seen in one blood specimen.

Epidemiological Findings in Ehima-Ken - The area selected for the survey in this Prefecture lay in the mountains at the present rail head of Yoshinobu, a good hour by train from Uwajima. The area lies along the Hiromi-gawa and other rivers. Most of the communities lie near water and like Tokushima the inhabitants seek fish and also fresh-water crabs as a protein supplement to their diet.

Although filariasis and malaria were rumored to be endemic in this lower portion of Ehime Ken neither the records which became available nor the amount of lymphadenopathy and/or splenomegaly which was encountered supported this view. Consequently, blood specimens were not collected.

It was reported locally that a certain amount of paragonimiasis existed. As stool examinations supported this, considerable attention was devoted to the diagnosis of this disease by sputum and by stool, and to securing further epidemiological data. Since the 83 persons examined (30 percent) were positive for paragonimiasis it appears that this entire area must be regarded as being endemic for paragonimiasis. The sputum and extra stools of a number of people were examined from four communities. The results of these findings appear in Table XX. Unfortunately it was inconvenient and well nigh impossible to secure 12 or 24 hour sputum samples. One interesting finding is the comparison of the number of persons who were positive by sputum and also by stool. This suggests that in chronic cases such as these a diagnosis by stool is more likely to be positive than

by sputum alone. However, it must be kept in mind that 12 or 24 hour sputum samples were not available. These findings are of interest because it is stated in the literature that only 40 percent of the cases show eggs in the stool. If this ratio holds it may prove to be attributable to the use of concentration techniques like the AMS III and the MGL. Further work is needed on this point.

A number of potential intermediate crab hosts were gathered from various areas in the vicinity and examined for the encysted metacercariae of Paragonimus. Unfortunately only a few specimens of the large, edible, migratory crab, Eriocheir japonicus, were available, since it was too early in the season for them to have migrated up from the ocean and bays. An attempt was also made to secure snails of the first intermediate host, Melania sp. These were crushed but no infections were found.

From these data it is evident that this region must be regarded as an endemic center of paragonimiasis. All persons using crabs as food should be educated to properly cook them first. This is the simplest and most effective method of rendering the encysted parasites harmless without destroying food value.

The overall incidence of intestinal parasites in this area of Ehime-Ken was 94.2 per cent (Table XXI). Of these 26.2 per cent harbored protozoa and 94.2 per cent helminths. The incidence of protozoa was somewhat lower than in the other areas surveyed. Perhaps the most striking finding was the presence of metagonimiasis in 12 per cent of the population examined.

Summary: The epidemiological survey of Shikoku revealed a number of interesting points. While the overall incidence of parasitism was high the rates for ascariasis trichoriasis and others were not as high as in some other surveys. There were fewer multiple infections, and when present, not as many different species were involved as in other areas. The rates for E. histolytica were high enough in some communities to suggest excessive contamination. No evidence of malaria or schistosomiasis was encountered. A single case of filariasis was found. Paragonimiasis is endemic in the Yoshinobu area of Ehime-Ken. Both fresh water crabs and snails were examined for the larval stages of Paragonimus sp.; some were found.

Table XX. Incidence of Paragonimiasis in Ehime-Ken on the Basis of Sputum and Stool Examination

Community	No. Examined	No. Positive by sputum Spec. #1	Spec. #2	No. Positive by Stool Spec. #1	Spec. #2	Total Positive No.	%
Yoshinobu-mura	22	2	3	3	3	3	13.6
Chikanaga-machi	26	3	2	-	4	6	23.0
Izumi-mura	17	5	6	6	6	8	47.0
Matsumaru-machi	18	-	5	1	7	8	44.4
Totals	83	10	16	10	20	25	30.1

EPIDEMIOLOGIC SURVEY OF HOKKAIDO: Introduction - Since Hokkaido represents a distinctive portion of the land area of Japan, and because of its geographical location, climatological conditions and sparseness of population (the total population approximates that of Tokyo) a survey for intestinal worms and protozoa, as well as blood parasites where indicated, was recognized to be of special interest. Since a moderate amount of hookworm had been encountered in Aomori Prefecture at the northern limits of Honshu (20), it was of special interest to determine how far hookworm infections extended into Hokkaido. Very high infection rates of Trichostrongylus sp. had been found in Aomori Prefecture, so it was also of interest to determine its prevalence in Hokkaido. Because of the numerous trout and salmon streams in Hokkaido the possibility of infections with Diphylobothrium latum was of concern, especially as this parasite had been reported by the Japanese. Geographic distribution of the survey is indicated in Figure 13.

Table XXI. Summary of the Incidence of Intestinal Parasites in Ehime-Ken

Character of Community	YOSHINOBU-MURA Yoshino-buraku Farming		CHIKANGA-MACHI Farming		IZUMI-MURA Farming		MATSUMARU-MACHI Urban & Farming		TOTAL	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. examined	104		104		102		103		413	
No. parasitized	98	94.2	99	95.4	94	92.2	98	95.1	389	94.2
No. with protozoa	21	20.1	15	14.4	39	38.2	33	32.0	108	26.2
No. with helminths	98	94.2	98	94.3	94	92.2	98	95.1	388	94.2
No. with										
E. histolytica	5	4.8	3	2.9	9	8.8	4	3.8	21	5.1
E. coli	9	8.6	7	6.7	29	28.4	23	22.3	68	16.5
Endolimax nana	8	7.6	5	4.8	16	15.7	13	12.6	42	10.2
Giardia lamblia	4	3.8	4	3.8	4	3.9	4	3.8	16	3.7
No. with										
Ascaris	67	64.4	79	76.0	78	76.4	87	84.4	311	75.6
Whipworm	63	60.5	74	71.3	65	63.8	77	74.7	279	67.6
Hookworm	60	57.6	47	45.3	41	40.3	47	45.6	195	47.4
Trichostrongylus sp.	2	1.9	1	0.9	1	1.0	-		4	1.0
Clonorchis sinensis	-	-	2	1.9	-	-	-	-	2	0.5
Heterophyes sp.	2	1.9	-	-	7	6.9	3	2.9	12	2.9
Metagonimus sp.	16	15.3	7	6.7	21	20.6	8	7.7	52	12.4

Findings - Throughout the Hokkaido survey the only helminths commonly encountered were four nematodes; ascaris, whipworm, *Trichostrongylus* sp. and pinworm (Table XXII). As might be expected, the rate of infection for hookworm was low. Tapeworm and fluke infections occurred only occasionally, there being only 9 cases of *Clonorchis sinensis*, 2 of *Metagonimus yokogawai*, 2 of *Diphylobothrium* sp., and 2 of *Hymenolepsis nana*.

Of the 2212 individuals examined 87.5 per cent were found to be parasitized, 81.0 per cent harbored helminths, and protozoan infections were found in 48.3 percent. At Odori-Ku only 57.0 per cent harbored helminths while in the Ainu village at Shizunai 96.4 per cent were so infected. These two figures represent the extremes of helminth infections. In the case of the protozoa the lowest rate, 31.0 percent, occurred in the upland area at Kagura, while the highest rate was found among the Ainu, 65.1 per cent of whom carried protozoa.

Considering the specific parasites, ascaris occurred in 68.9 per cent of the total number examined. The incidence ranged from 31.2 per cent in Odori-Ku, Sappore, to 93.3 per cent in the Ainu village. Other low rates were found in Obihiro City and the upland farming area at Kagura.

For the entire survey, the incidence of whipworm was 29.3 per cent. The highest figure (57.0 percent) was encountered at Onakayama near Hakodate while the lowest, approximately 15.0 percent, was found in several other places. The average incidence of 29.3 per cent for whipworm is quite in contrast to that found in Aomori (most northern prefecture of Honshu) where some of the highest rates thus far encountered in our surveys occurred. In fact, at Ominata the incidence of whipworm surpassed that of ascaris.

Hookworm was virtually absent from Hokkaido as may be seen by the fact that only 1.9 per cent of the persons examined harbored this parasite. Considering climatic conditions, even this rate may rightfully be considered high. It is possible that an analysis of the data will show that many of these infections were contracted elsewhere (other parts of Japan, Okinawa and the South Pacific). However, an interesting exception was noted at Hamatonbetsu, near Wakkanai (which is located at the northern tip of Hokkaido) where an incidence of 7.5 per cent was found. Almost without exception, the parasite density index was very low.

Figure 13


Wakkanai
Toyotomi
Hamatonbetsu
SOYA
Soruru
Setsushi
RUMOE
Kagura
ABASHIRI
KAMIKAWA
SORACHI
Bibai
NEMURO
KUSHIRO
KASAI
Akan
Nemuro
Akkeshi
Sapporo
ISHIKARI
Obihiro
HIDAKA
URAKAWA
HOKKAIDŌ
HIYAMA
OSHIMA
LEGEND
Communities surveyed
SCALE OF MILES
0 20 40 60 80 100

Table XXII. Summary of Principle Parasites in Hokkaido

Area / Community	Iwamizawa Bibai		Wakkanai Wakkanai		Wakkanai Toyotomi		Wakkanai Hamatombetsu	
Character of Area	Mining		Urban		Farming		Farming	
	No.	%	No.	%	No.	%	No.	%
No. Examined	115		70		125		107	
No. Parasitized	107	93.0	62	88.6	110	88.0	57	90.7
No. with Helminths	106	92.2	60	85.7	101	80.8	85	79.4
No. with Protozoa	46	40.0	26	37.1	60	48.0	58	54.2
Helminths								
Ascaris	98	85.2	47	67.1	95	76.0	64	59.8
Trichuris trichiura	48	41.7	28	40.0	26	20.8	44	41.1
Hookworm	3	2.6	3	4.3	1	0.8	8	7.5
Trichostrongylus sp.	23	20.0	9	12.9	19	15.2	31	29.0
E. vermicularis*	6/24	25.0	6/11	54.5	23/41	56.1	5/12	41.7
Protozoa								
E. histolytica	6	5.2	9	12.9	7	5.6	14	13.1
E. coli	30	26.1	16	22.9	45	36.0	35	32.7
E. nana	18	15.7	8	11.4	27	21.6	27	25.2
Giardia lamblia	7	6.1	1	1.4	5	4.0	11	10.3
Iodamoeba butschlii	1	0.9	0	0.0	3	2.0	2	1.9

Area / Community	Monbetsu Saruru		Monbetsu Nishiokappe		Asahigawa Kagura (Valley)		Asahigawa Kagura (Upland)	
Character of Area	Fishing		Farming		Farming		Farming	
	No.	%	No.	%	No.	%	No.	%
No. Examined	124		121		124		126	
No. Parasitized	106	85.5	104	86.0	98	79.0	90	71.4
No. with Helminths	98	79.0	90	74.4	83	66.9	78	61.9
No. with Protozoa	67	54.0	56	46.3	63	50.8	39	31.0
Helminths								
Ascaris	83	66.9	78	64.5	59	47.6	51	40.5
Trichuris trichiura	43	34.7	19	15.7	21	16.9	25	19.8
Hookworm	2	1.6	0	0.0	0	0.0	3	2.4
Trichostrongylus sp.	16	12.9	24	19.8	24	19.8	29	23.0
E. vermicularis*	14/30	46.7	6/14	42.9	9/21	42.9	4/15	26.7
Protozoa								
E. histolytica	17	13.7	18	14.9	5	4.0	10	7.9
E. coli	52	41.9	36	29.8	38	30.6	20	15.9
E. nana	25	20.2	22	18.2	24	19.4	13	10.3
Giardia lamblia	13	10.5	2	1.6	15	12.1	10	7.9
Iodamoeba butschlii	0	0.0	4	3.3	2	1.6	1	0.8

Table XXII. Continued

Area	Obihiro				Sapporo			
Community	Obihiro		Nakubetsu		Odori-ku		Toyohira-ku	
Character of Area	Urban		Farming		Urban		Urban	
	No.	%	No.	%	No.	%	No.	%
No. Examined	104		122		93		114	
No. Parasitized	86	82.7	108	88.5	63	67.7	94	82.5
No. with Helminths	72	69.2	94	77.0	53	57.0	82	71.9
No. with Protozoa	51	49.0	62	50.8	33	35.5	51	44.7
Helminths								
Ascaris	46	44.2	89	73.0	29	31.2	57	50.0
Trichuris trichiura	23	22.1	18	14.8	18	19.4	22	19.9
Hookworm	2	1.9	1	0.8	1	1.1	2	1.8
Trichostrongylus sp.	22	21.2	23	18.9	4	4.3	13	11.4
*E. vermicularis**	6/18	33.3	15/27	55.6	12/22	54.5	18/27	66.7
Protozoa								
E. histolytica	10	9.6	13	10.7	5	5.4	1	0.9
E. coli	22	21.2	38	31.1	22	23.7	37	32.5
E. nana	33	31.7	32	26.2	15	16.1	15	13.2
Giardia lamblia	6	5.8	5	4.1	6	6.5	7	6.1
Iodamoeba butschlii	0	0.0	8	6.6	1	1.0	6	5.3

Area	Kushiro				Nemuro			
Community	Akan		Akkeshi		Nemuro		Nemuro	
Character of Area	Farming		Fishing		Farming		Fishing	
	No.	%	No.	%	No.	%	No.	%
No. Examined	125		105		122		102	
No. Parasitized	117	93.6	99	94.3	110	90.2	99	91.2
No. with Helminths	116	92.8	97	92.4	109	89.3	85	83.3
No. with Protozoa	62	49.6	65	61.9	55	45.1	52	51.0
Helminths								
Ascaris	112	89.6	91	86.7	104	85.2	68	66.7
Trichuris trichiura	24	19.2	35	33.3	38	31.1	26	25.5
Hookworm	3	2.4	1	1.0	0	0.0	2	2.0
Trichostrongylus sp.	38	30.4	14	13.3	5	4.1	31	30.4
*E. vermicularis**	2/23	41.4	14/26	53.8	7/19	36.8	7/20	35.0
Protozoa								
E. histolytica	11	8.8	12	11.4	10	8.2	13	12.7
E. coli	35	28.0	51	48.6	40	32.8	33	32.4
E. nana	37	29.6	22	21.0	19	15.6	21	20.6
Giardia lamblia	7	5.5	14	13.3	6	4.9	7	6.9
Iodamoeba butschlii	8	6.4	2	1.9	8	6.5	2	2.0

Table XXII Continued

Area / Community	Shizunai Ainu Tribe		Hokodate Nanae		Hokodate Onakayama		TOTAL	
Character of Area	Farming No.	%	Farming No.	%	Farming No.	%	No.	%
No. Examined	195		111		107		2212	
No. parasitized	190	97.4	102	91.9	99	92.5	1935	87.5
No. with Helminths	188	96.4	97	87.4	97	90.7	1791	81.0
No. with Protozoa	127	65.1	54	48.6	42	39.3	1069	48.3
Helminths								
Ascaris	182	93.3	89	80.2	83	77.6	1525	68.9
Trichuris trichiura	84	43.1	45	40.5	61	57.0	648	29.3
Hookworm	0	0.0	1	0.9	8	7.5	41	1.9
Trichostrongylus sp.	22	11.3	11	9.9	15	14.0	373	16.9
E. vermicularis*	20/38	52.6	12/30	40.0	14/30	46.7	210/454	46.3
Protozoa								
E. histolytica	51	26.2	8	7.2	4	3.7	224	10.1
E. coli	95	48.7	37	33.3	34	31.8	716	32.4
E. nana	68	34.9	21	18.9	23	21.5	470	21.2
Giardia lamblia	8	4.1	6	5.4	1	0.9	137	6.2
Iodamoeba butschlii	2	1.0	4	3.6	1	0.9	55	2.5

As in the case of whipworm the rates for Trichostrongylus sp.were low in contrast to those in Aomori prefecture. The average rate in Hokkaido was only 16.9 per cent while the school children at Tsuruda in Aomori yielded over 80 per cent infection. The infection rate was 46.3 per cent in the 454 children who were examined for pinworm by means of the Graham scotch tape swab. Interestingly enough, the highest incidence was found in Sapporo, one of the most modern urban communities on the island.

Endamoeba histolytica was found in 10.1 per cent of the total individuals examined. The incidence of 26.2 per cent found among the Ainu at Shizunai was the highest rate which has thus far been encountered in the Far East on our surveys. Excluding the Ainu, the incidence on Hokkaido was 8.2 per cent. The infection by Endamoeba coli was 32.4 per cent and Endolimax nana 21.2 per cent.

In addition to the extraordinary protozoan rates encountered in the Ainu, those obtained at Kagura (upland area) were also considered unusual. They were unusual in that they were low, in spite of the fact that the drinking water was obtained chiefly from the irrigation ditches which ran along the edge of the rice paddies. In the absence of any other compensating factor, this condition might have accounted for extremely high infection rates for protozoa. Careful interrogation brought out the fact that the people made a policy of boiling their drinking water; however, it was admitted in most households questioned that during busy seasons this practice was not followed so dilligently. During the winter they relied quite extensively on melted snow. Since the people were not consistent in their policy of boiling the water it would hardly be justified to explain the low protozoan rates on this basis, but at least this probably was an important factor in reducing the threat of amebiasis from drinking water.

In the Ainu village the water supply was secured almost entirely from open wells which were lined with wood. This lining extended about 6 inches above the surface of the ground and furnished some protection. Although the wells were not the best, the water supply appeared moderately good. One source of contamination was observed. The mild cans were lowered into the wells for cooling, thus affording an opportunity for the introduction of surface soil. General innudation of the valley and flooding of wells occurred during the fall of 1947. This also might have been a factor in bringing about an abnormally high incidence of amebiasis. Other floodings, however, had not occurred

in the memory of the people questioned. Although the living conditions of the Ainu are relatively poor they were not sufficiently different from those of Japanese to account for the marked difference in protozoan infections that were encountered.

Parasitic Infection Correlated with Occupation - The various population centers studied were listed on the basis of the principal occupation, and a comparison of the parasitic findings in the several categories was made (Table XXIII). The Ainu village was treated separately because of the unusual findings which were encountered there. The mining data were also listed separately because these did not fall into any of the other categories. One or more helminths were found in 79.9, 83.3 and 70.1 per cent in farming, fishing and urban populations respectively. Although limited in degree, the lesser rate found in urban populations surely represented a significant difference. The findings for ascaris and Trichostrongylus sp. were compatible with those above inasmuch as they were similar in the farming and fishing communities, and in urban populations. In the case of whipworm the rates were similar in the farming and urban categories and noticeably higher in the fishing communities.

The protozoan infections, collectively, for farming urban, and fishing populations were correspondingly 45.5, 42.3 and 55.3 per cent. In the case of specific organisms, E. histolytica and E. coli were equally more prevalent in the fishing populations whereas E. nana had a range limited to only 2.0 per cent for all three population categories.

Summary - A general description is given of the population centers which were studied. The commonly encountered helminths included: ascaris, whipworm, Trichostrongylus sp. and pinworm. Hookworm infections, in general, were rare, but in one community near the northern end of the island an incidence of 7.5 per cent was encountered. Tapeworm and fluke infections were seldom detected. The incidence of the helminths in general was relatively low. Ascaris was high in many areas, but unusually low in others, averaging 68.9 per cent for the island. Whipworm and Trichostrongylus sp. were noticeably lower than adjacent areas in northern Honshu while the pinworm rate, 46.3 per cent was not unusual. The incidence of Endamoeba histolytica, E. coli and Endolimax nana were correspondingly 10.1, 32.4 and 21.2 per cent. An extraordinarily high incidence of 26.2 per cent for E. histolytica was found in the Ainu Village at Shizunai.

Table XXIII. Comparison of Parasitic Infections on Basis of Chief Occupational Pursuits

	Farming		Urban		Fishing		Mining		Ainu		Total
	No.	%	No.	%	No.	%	No.	%	No.	%	
No. Examined	1,083		381		438		115		195		2,212
No. Parasitized	938	86.6	305	80.1	395	90.2	107	93.0	190	97.4	1,935
No. with Helminths	865	79.9	267	70.1	365	83.3	106	92.2	188	96.4	1,791
No. with Protozoa	493	45.5	161	42.3	242	55.3	46	40.2	127	65.1	1,069
Ascaris	760	70.2	179	47.0	306	69.9	98	85.2	182	93.3	1,525
Whipworm	277	25.6	91	23.9	148	33.8	48	41.7	84	43.1	648
Trichostrongylus sp.	188	17.4	48	12.6	92	21.0	23	20.0	22	11.3	373
Endamoeba histolytica	86	7.9	25	6.6	56	12.8	6	5.2	51	26.2	224
E. coli	823	29.8	97	25.5	171	39.0	30	26.1	95	48.7	716
Endolimax nana	218	20.1	71	18.6	95	21.7	18	15.7	68	34.9	470

EPIDEMIOLOGIC SURVEY OF OKINAWA: Introduction - There was little information available on the situation in Okinawa, one of the Ryukyus, other than TB MED 108 (21). On Okinawa the military installations were located in close proximity to the natives. Mixed with these natives were groups of Filipinos. In order to obtain up-to-date information on this situation it was decided to dispatch a survey team to Okinawa during the summer of 1949. This group was charged with the responsibility of securing data on the presence of intestinal parasites, both protozoan and helminths, as well as on the current incidence of malaria and filariasis. The team also planned to

secure information on whether or not such diseases as schistosomiasis, clonorchiasis and paragonimiasis were indigenous to this island. Additional data were to be sought on the prevalence of Japanese B encephalitis, scrub typhus and related diseases.

Findings on Intestinal Parasites on Okinawans - The stool of a total of 2145 persons were examined from the nine districts for the presence of intestinal worms and protozoa. Of these 92 per cent were parasitized, 90 per cent having helminths and 41 per cent protozoa (See Table XXIV). The over-all incidence as reported here is comparable to the data from Japan which ranges in our surveys from a low of 87 per cent to a peak of 99 per cent with most regions falling in the 92-95 per cent range. In many areas in Japan the incidence for both worms and protozoa was slightly higher than on Okinawa.

As noted above approximately 90 per cent of the 2145 persons examined carried helminths. Hookworm appeared as the most prevalent worm being found in 72 per cent of the individuals examined. This was very interesting in view of the results obtained in the other surveys. In Japan the incidence of nookworm ranged between 13 and 72 per cent while in Korea it was 45 per cent. In Okinawa hookworm was the dominant parasite while in all other regions studied it almost always ranged below ascaris and whipworm in incidence. The importance of hookworm on this island is shown by the index of parasite density which was 82, compared with all of our other observations in which the density index was above 60 only in Okayama and Kagoshima prefecture and in Korea. It is known that the single most important crop is sweet potatoes which is grown on dry fields. Soil conditions and the growing of sweet potatoes are quite ideal for the survival of hookworm larvae. These conditions associated with the well-nigh universal habit of going bare foot in the fields fosters the acquisition of this parasite. The situation is quite comparable to some areas of China where hookworm constitutes a serious problem among the farmers raising sweet potatoes (22). Evidence of clinical hookworm disease was found but rarely.

Ascaris lumbricoides is not the dominant parasite it is in Japan and was encountered in only 49 per cent of the 2145 Okinawans who were examined. Persons infected by Ascaris usually carried a considerable number of worms. This was shown by the parasite density index of 172. Only in Kagoshima prefecture and in Korea was the worm burden higher for Ascaris.

However, whipworm, with only 21 percent, assumed a much less important position as its index was only 26. This figure is considerably less than any of the areas in Japan. It is evident that conditions were not particularly favorable for the survival of this parasite on Okinawa.

The summary of the parasite density index by districts appears in Table XXV.

Strongyloides stercoralis was found in about 13 per cent of the Okinawans examined. This is interesting as it constitutes the highest incidence encountered in any of our surveys. It is believed that soil and other environmental conditions that would favor hookworm would also favor the survival and development of the larvae of Strongyloides. This is substantiated in part by the apparent correlation between the incidence of hookworm and Strongyloides in the various districts of Okinawa.

Trichostrongylus sp. which was found in considerable numbers in some areas of Japan was conspicuous by its virtual absence on Okinawa. It was present in less than 1 per cent of the people. This tends to support the view held by some that this parasite is more typical of the cooler areas, or at least regions having a definite winter season or the equivalent.

Data on the other parasites will be found in Table XXIV.

Table XXIV. Summary of Intestinal Parasites in Okinawan Natives
Made in July - August, 1949

	Hentona District							
	Ginama		Aha		Oganeku		Taira	
	No	%	No.	%	No.	%	No.	%
No. of persons examined	101		123		102		112	
No. with one or more parasites	88	87.1	123	100	89	87.3	108	96.4
No. with helminths	86	85.1	123	100	89	87.3	108	96.4
No. with protozoa	30	29.7	18	14.6	29	28.4	83	47.3
Helminths:								
Ascaris lumbricoides	35	34.7	76	61.8	61	59.8	55	49.1
Trichuris trichiura	6	5.9	13	10.6	19	18.6	17	15.2
Hookworm	71	70.3	113	91.9	74	72.5	87	77.7
Trichostrongylos sp.	0	0.0	2	1.6	0	0.0	1	0.9
Enterobius vermicularis in stool	1	1.0	6	4.9	6	5.9	4	3.6
Strongyloides stercoralis	5	5.0	17	13.8	14	13.7	13	11.6
Clonorchis sinensis	0	0.0	3	2.4	0	0.0	2	1.8
Metagonimus yokogawai	0	0.0	0	0.0	0	0.0	0	0.0
Taenia sp.	0	0.0	0	0.0	0	0.0	0	0.0
Schistosoma sp.	0	0.0	0	0.0	0	0.0	0	0.0
Hymenolepis nana	1	1.0	0	0.0	0	0.0	0	0.0
Hymenolepis diminuta	1	1.0	0	0.0	0	0.0	0	0.0
Echinostoma sp.	0	0.0	0	0.0	0	0.0	0	0.0
Paragonimus westermani	0	0.0	0	0.0	0	0.0	0	0.0
Trematode unidentified	0	0.0	0	0.0	0	0.0	0	0.0
Heterophyid	0	0.0	0	0.0	0	0.0	0	0.0
Enterobius vermicularis in swab	8/25	32.0	9/17	52.9	8/12	25.0	13/27	48.1
Protozoa:								
Endamoeba histolytica	9	8.9	3	2.4	7	6.9	22	19.6
Endamoeba coli	9	8.9	12	9.8	12	11.8	22	19.6
Endolimax nana	21	20.8	2	1.6	23	22.5	35	31.3
Iodamoeba butschlii	2	2.0	0	0.0	3	2.9	4	3.6
Giardia lamblia	4	4.0	4	3.3	5	4.9	6	5.4
Chilomastix mesnili	2	2.0	1	0.8	0	0.0	0	0.0

Table XXIV. Continued

	Taira District				Ginoza District			
	Nakao		Nakajin		Motobu		Henoka	
	No.	%	No.	%	No.	%	No.	%
No. of persons examined	100		100		102		95	
No. with one or more parasites	93	93.0	96	96.0	94	90.2	85	89.5
No. with helminths	92	92.0	96	96.0	92	90.2	82	86.3
No. with protozoa	39	39.0	30	30.0	47	46.1	39	41.1
Helminths:								
Ascaris lumbricoides	78	78.0	55	55.0	64	62.7	49	51.6
Trichuris trichiura	25	25.0	14	14.0	24	23.5	17	17.9
Hookworm	60	60.0	83	83.0	72	70.6	68	71.6
Trichostrongylos sp.	0	0.0	1	1.0	0	0.0	0	0.0
Enterobius vermicularis in stool	2	2.0	1	1.0	0	0.0	4	4.2
Strongyloides stercoralis	25	25.0	9	9.0	10	9.4	30	31.6
Clonorchis sinensis	1	1.0	1	1.0	0	0.0	0	0.0
Metagonimus yokogawai	0	0.0	0	0.0	0	0.0	0	0.0
Taenia sp.	0	0.0	0	0.0	0	0.0	0	0.0
Schistosoma sp.	0	0.0	0	0.0	0	0.0	0	0.0
Hymenolepis nana	0	0.0	1	1.0	1	1.0	0	0.0
Hymenolepis dimimuta	0	0.0	0	0.0	0	0.0	0	0.0
Echinostoma sp.	0	0.0	0	0.0	0	0.0	0	0.0
Paragonimus westermani	0	0.0	0	0.0	0	0.0	0	0.0
Trematode unidentified	0	0.0	1	1.0	25	24.5	0	0.0
Heterophyid	1	1.0	0	0.0	0	0.0	1	1.1
Enterobius vermicularis in swab	2/22	9.1	0	0.0	5/24	20.8	7/24	29.2
Protozoa:								
Endamoeba histolytica	8	8.0	6	6.0	21	20.6	6	6.3
Endamoeba coli	15	15.0	8	8.0	26	25.5	19	20.0
Endolimax nana	27	27.0	20	20.0	27	26.5	17	17.9
Iodamoeba butschlii	0	0.0	0	0.0	0	0.0	0	0.0
Giardia lamblia	6	6.0	4	4.0	5	4.9	8	8.4
Chilomastix mesnili	1	1.0	0	0.0	0	0.0	0	0.0

Table XXIV. Continued

	Ishikawa Dist.				Maebaru Dist.			
	Afuso		Ishikawa		Gushikawa		Tomari	
	No	%	No.	%	No.	%	No.	%
No. of persons examined	100		66		100		105	
No. with one or more parasites	91	91.0	60	90.9	95	95.0	93	88.6
No. with helminths	90	90.0	58	87.9	91	91.0	91	86.7
No. with protozoa	36	36.0	38	57.6	48	48.0	59	56.2
Helminths:								
Ascaris lumbricoides	60	60.0	44	66.7	31	31.0	14	13.3
Trichuris trichiura	9	9.0	11	16.7	31	31.0	18	17.1
Hookworm	69	69.0	46	69.7	84	84.0	78	74.3
Trichostrongylos sp.	9	9.0	3	4.5	0	0.0	1	1.0
Enterobius vermicularis in stool	0	0.0	0	0.0	1	1.0	1	1.0
Strongyloides stercoralis	0	0.0	9	13.6	20	20.0	22	21.0
Clonorchis sinensis	0	0.0	0	0.0	0	0.0	0	0.0
Metagonimus yokoagawai	1	1.0	0	0.0	0	0.0	0	0.0
Taenia sp.	0	0.0	0	0.0	0	0.0	1	1.0
Schistosoma sp.	0	0.0	0	0.0	0	0.0	1*	1.0
Hymenolepis nana	0	0.0	0	0.0	0	0.0	0	0.0
Hymenolepis diminuta	0	0.0	1	1.5	0	0.0	0	0.0
Echinostoma sp.	0	0.0	0	0.0	0	0.0	0	0.0
Paragonimus westermani	0	0.0	0	0.0	0	0.0	0	0.0
Trematode unidentified	0	0.0	0	0.0	0	0.0	0	0.0
Heterophyid	0	0.0	0	0.0	1	1.0	0	0.0
Enterobius vermicularis in swab	3/19	15.8	6/20	30.0	5/22	22.7	3/17	17.6
Protozoa:								
Endamoeba histolytica	8	8.0	14	21.2	14	14.0	22	20.1
Endamoeba coli	18	18.0	20	30.3	29	29.0	28	26.7
Endolimax nana	15	15.0	28	42.4	20	20.0	35	33.3
Iodamoeba butschlii	1	1.0	2	3.0	2	2.0	2	1.9
Giardia lamblia	6	6.0	9	13.6	2	2.0	9	8.6
Chilomastix mesnili	3	3.0	0	0.0	3	3.0	1	1.0

* Schistosoma mansoni

Table XXIV. Continued

	Koza Dist.						Naha Dist.			
	Kadena		Tobaru		Jagaru		Naha		Shuri	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. of persons examined	107		102		100		86		82	
No. with one or more parasites	100	93.5	91	89.2	97	97.0	75	87.2	75	91.5
No. with helminths	94	87.9	88	86.3	97	97.0	71	82.6	75	91.5
No. with protozoa	55	51.4	34	33.3	44	44.0	39	45.3	36	43.9
Helminths:										
Ascaris lumbricoides	64	59.8	53	52.0	35	35.0	42	48.8	55	67.1
Trichuris trichiura	15	14.0	22	21.6	39	39.0	28	32.6	31	37.8
Hookworm	76	71.0	67	65.7	81	81.0	53	61.6	44	53.7
Trichostrongylos sp.	0	0.0	0	0.0	0	0.0	0	0.0	3	3.7
Enterobius vermicularis in stool	0	0.0	2	2.0	3	3.0	1	1.2	2	2.4
Strongyloides stercoralis	19	17.8	5	4.9	12	12.0	6	7.0	4	4.9
Clonorchis sinensis	0	0.0	2	2.0	0	0.0	2	2.3	0	0.0
Metagonimus yokogawai	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0
Taenia sp.	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0
Schistosoma sp.	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Hymenolepis nana	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Hymenolepis diminuta	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Echinostoma sp.	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Paragonimus westermani	1	0.9	0	0.0	0	0.0	0	0.0	0	0.0
Trematode unidentified	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Heterophyid	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Enterobius vermicularis in swab	3/25	12.0	3/27	11.1	14/29	48.3	3/15	20.0	5/15	33.3
Protozoa:										
Endamoeba histolytica	16	15.0	11	10.8	15	15.0	17	19.8	20	24.4
Endamoeba coli	21	19.6	16	15.7	19	19.0	18	20.9	16	19.5
Endolimax nana	33	30.8	10	9.8	21	21.0	13	15.1	19	23.2
Iodamoeba butschlii	2	1.9	4	3.9	2	2.0	2	2.3	0	0.0
Giardia lamblia	15	14.0	8	7.8	4	4.0	8	9.3	6	7.3
Chilomastix mesnili	1	0.9	0	0.0	0	0.0	0	0.0	2	2.4

Table XXIV. Continued

	Itoman Dist.						Chinen Dist.			
	Itoman		Takamine		Kiyamu		Haebaru		Tamagusuku	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. of persons examined	84		86		105		71		116	
No. with one or more parasites	71	84.5	73	84.9	96	91.4	69	97.2	109	94.0
No. with helminths	68	81.0	71	82.6	93	88.6	67	94.4	107	92.2
No. with protozoa	33	39.3	42	48.8	56	53.3	27	38.0	46	39.7
Helminths:										
Ascaris lumbricoides	29	34.5	19	22.1	45	42.9	52	73.2	29	25.0
Trichuris trichiura	31	36.9	10	11.6	14	13.3	25	35.2	26	22.4
Hookworm	40	47.6	61	70.9	81	77.1	44	62.0	102	87.9
Trichostrongylos sp.	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Enterobius vermicularis in stool	0	0.0	0	0.0	3	2.9	2	2.8	1	0.9
Strongyloides stercoralis	14	16.7	13	15.1	24	22.9	5	7.0	10	8.6
Clonorchis sinensis	0	0.0	0	0.0	0	0.0	0	0.0	2	1.7
Metagonimus yokogawai	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Taenia sp.	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Schistosoma sp.	0	0.0	1**	1.2	0	0.0	0	0.0	0	0.0
Hymenolepis nana	0	0.0	1	1.2	1	1.0	0	0.0	0	0.0
Hymenolepis diminuta	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0
Echinostoma sp.	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0
Paragonimus westermani	0	0.0	0	0.0	0	0.0	0	0.0	2	1.7
Trematode unidentified	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Heterophyid	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Enterobius vermicularis in swab	7/26	26.9	9/19	47.4	16/28	57.1	15/30	50.0	7/22	31.8
Protozoa:										
Endamoeba histolytica	16	19.0	16	18.8	26	24.8	4	5.6	10	8.6
Endamoeba coli	20	23.8	21	24.4	41	39.0	16	22.5	25	21.6
Endolimax nana	8	9.5	25	29.1	29	27.6	8	11.3	22	19.0
Iodamoeba butschlii	2	2.4	2	2.3	10	9.5	1	1.4	0	0.0
Giardia lamblia	4	4.8	5	5.8	6	5.7	4	5.6	7	6.0
Chilomastix mesnili	1	1.2	0	0.0	1	1.0	4	5.6	0	0.0

** *Schistosoma japonicum*

Table XXIV. Continued

	No.	%
No. of persons examined	2,145	
No. with one or more parasites	1,971	91.9
No. with helminths	1,929	89.9
No. with protozoa	878	40.9
Helminths:		
Ascaris lumbricoides	1,045	48.7
Trichuris trichiura	445	20.7
Hookworm	1,554	72.4
Trichostrongylos sp.	20	0.9
Enterobius vermicularis in stool	40	1.9
Strongyloides stercoralis	286	13.3
Clonorchis sinensis	13	0.6
Metagonimus yokogawai	2	0.1
Taenia sp.	2	0.1
Schistosoma sp.	2	0.1
Hymenolepis nana	5	0.2
Hymenolepis diminuta	3	0.1
Echinostoma sp.	1	0.046
Paragonimus westermani	3	0.1
Trematode unidentified	26	1.2
Heterophyid	3	0.1
Enterobius vermicularis in swab	151/485	31.1
Protozoa:		
Endamoeba histolytica	291	13.6
Endamoeba coli	431	20.1
Endolimax nana	458	21.4
Iodamoeba butschlii	41	1.9
Giardia lamblia	135	6.3
Chilomastix mesnili	20	0.9

Table XXV. Summary of Parasite Density Index for Okinawa

	Ascaris	Whipworm	Hookworm	Trichostrongylus
Okinawa	172	26	82	23
Hantona	171	20	93	
Taira	207	25	82	
Ginoza	298	54	101	
Ishikawa	156	32	84	
Maebaru	110	23	78	
Koza	131	21	60	
Naha	216	30	90	
Itoman	102	24	47	
Chinen	163	28	131	

The overall incidence of protozoa was not unusually high. However, Okinawa with 13.6 per cent has one of the highest incidences of Endamoeba histolytica yet encountered. The localized area occupied by an Ainu tribe in Hokkaido has been the only area with a higher incidence. Considering larger areas only Yamanashi and Fukui prefectures with 10.3 and 11 per cent respectively approached it. All of the other areas studied fell between 3 and 6 per cent. On Okinawa there were several communities which yielded over 20 per cent E. histolytica. This indicates that in general the hygienic conditions were not good. Giardia lamblia with over 6 per cent was fairly high. The other non-parasitic protozoa fell within the normal range (see Table XXIV).

Malaria in Okinawans - This disease is prevalent in the Ryukyu Islands although historically it does not appear to be as important on Okinawa as on some of the neighboring islands (21). For example, on Ishagaki entire settlements have been wiped out. Such epidemics were reportedly caused by infections of Plasmodium falciparum. In April 1945 after the American troops had landed, it was feared that malaria might be a problem of serious proportions. Consequently the various malaria survey teams began intensive studies to determine the prevalence of this disease on the island. A total of 2209 slides were examined by 7 units and only 0.4 per cent positives were found (23). The highest rates in northern Okinawa were found in Ginoza, Sedake, and Taira where 1, 3, 8 and 10.9 per cent respectively were positive.

As a part of the current survey 1262 persons were examined for malaria. A total of 4.8 per cent were parasitized, nearly all being Plasmodium vivax (see Table XXVI). This figure indicates an increase in malaria when compared with 0.4 per cent secured four years previously. In certain districts where comparisons can be made the incidence appears to have shifted (see below).

Comparison of Results of Survey Made in 1945 and 1949

District	Okinawan Natives 1945		Okinawan Natives 1949	
	No. Examined	% Positive	No. Examined	% Positive
Ginoza	750	1.3	63	15.9
Taira	512	10.9	192	2.1

The map shows the incidence by communities (Fig.14). From this it is apparent that the northern half of the island has much more malaria than the southern half, 7.7 per cent compared with 2.7 per cent respectively. The districts where the highest rates occurred were Ginoza with 15.9 Hentona (Taira) with 15, and Ishikawa with 13.2 per cent. These first two districts are believed to have more paddies than many of the other regions. Another factor in the difference may well be the intensive efforts at mosquito control that have been instituted in the southern half because of the presence of numerous military installations on this part of the island. It is believed by many that the northern part was the original endemic area for malaria on Okinawa. In this connection it should be pointed out that the extent of splenomegaly and other findings suggest a much higher incidence than was recorded.

The chief vector is knows to be Anopheles hyrcanus sinensis. This mosquito breeds in the paddies. Consequently the conditions may well change as new paddies are developed in new areas of rice cultivation.

Filariasis in Okinawans - This disease was not recorded by TB MED 108 as being present in Okinawa although the statement was made that "The disease might be encountered in any of the islands". A total of 1262 native Okinawans were examined by the modified Knott Technique for microfilariae. One hundred and twenty one, or 9.6 per cent, were positive for Wucheria bancrofti. The incidence varied from 1.8 to 28.0 per cent in individual communities. Samples were taken in all of the 22 communities and infected persons were found in all. However, there was little clinical evidence of disease with only a few cases of elephantiasis being encountered.

The finding of microfilariae in the blood of persons from such widely scattered areas (Fig.14) is indicative of the endemicity of the disease on Okinawa. The common pest mosquito, Culex quinquefasciatus, is one of the good vectors of filariasis and it is worthy of note that this species is quite prevalent on the island.

Other Diseases - Attempts were made to secure data on the presence of schistosomiasis, clonorchiasis and paragonimiasis. It can be stated definitely that in the areas surveyed schistosomiasis is not endemic. The known snail hosts, Oncomelania quadrasi of the Philippines, O. hupensis of China, or O. nosophora of Japan, were not encountered. Local public health officials also have no record of the disease being established and only two cases (one of S. mansoni and one of S. japonicum) were encountered. Both of these gave histories of having been in known endemic centers for the disease elsewhere.

Clonorchiasis seems to be endemic in a few areas. However, infected snails were not encountered. Three cases of paragonimiasis were also encountered suggesting the possibility that this parasite may become established. Further survey work on these two diseases is indicated.

Table XXIV. summarizing the findings on the Okinawans lists the presence of 26 cases of unidentified trematode eggs. These closely resembled eggs of fish parasites and were probably passed through the digestive tract.

Japanese B encephalitis is known to occur on Okinawa and a total of 38 cases were reported in the period 1 June to 17 September. Three cases occurred in June and in occupation personnel. For additional information see Virus Section, this report. Scrub, flea-borne and louse-borne typhus are reported in the Ryukus by TB MED 108 but no cases were encountered.

Table XXVI. Summary of Blood Parasites in Okinawan Natives

	Hentona District							
	Ginama		Aha		Oganeku		Taira	
	No.	%	No.	%	No.	%	No.	%
Blood Parasites:								
Filaria								
No. examined	68		65		56		75	
No. with Wuchereria bancrofti	6	8.8	9	13.8	1	1.8	3	4.0
Malaria								
No. examined	70		73		62		80	
No. with parasites	8	11.5	2	2.7	3	4.8	12	15.0
Plasmodium vivax	8	11.5	2	2.7	3	4.8	12	15.0
Trophozoite unidentified	0	0.0	0	0.0	0	0.0	0	0.0

	Tairu Dist.				Ginoza Dist.			
	Nakao		Nakijin		Motobu		Henoko	
	No.	%	No.	%	No.	%	No.	%
Blood Parasites:								
Filaria								
No. Examined	55		66		57		59	
No. with Wuchereria bancrofti	4	7.3	3	4.5	1	1.8	8	13.6
Malaria								
No. examined	61		73		58		63	
No. with parasites	3	4.9	0	0.0	1	1.7	10	15.9
Plasmodium vivax	3	4.9	0	0.0	1	1.7	10	15.9
Trophozoite unidentified	0	0.0	0	0.0	0	0.0	0	0.0

Table XXVI. Continued

	Ishikawa Dist.				Maebaru Dist.			
	Afuso		Ishikawa		Gushikawa		Tomari	
	No.	%	No.	%	No.	%	No.	%
Blood Parasites:								
Filaria								
No. Examined	60		26		75		40	
No. with Wuchereria bancrofti	6	10.0	1	3.8	8	10.7	2	5.0
Malaria								
No. Examined	68		30		71		48	
No. with parasites	9	13.2	0	0.0	2	2.8	3	6.3
Plasmodium vivax	8	11.8	0	0.0	2	2.8	3	6.3
Trophozoite unidentified	1	1.4	0	0.0	0	0.0	0	0.0

	Koza Dist.						Naha Dist.			
	Kadena		Tobaru		Jagaru		Naha		Shuri	
	No.	%	No.	%	No.	%	No.	%	No.	%
Blood Parasites:										
Filaria										
No. examined	64		65		91		49		43	
No. with Wuchereria bancrofti	18	28.1	11	16.9	7	7.7	4	8.2	3	7.0
Malaria										
No. examined	86		75		90		54		50	
No. with parasites	5	5.8	2	2.7	2	2.2	0	0.0	0	0.0
Plasmodium vivax	4	4.7	2	2.7	2	2.2	0	0.0	0	0.0
Trophozoite unidentified	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0

	Itoman Dist.						Chinen Dist.			
	Itoman		Takamine		Kiyamu		Haebaru		Tamagusuku	
	No.	%	No.	%	No.	%	No.	%	No.	%
Blood Parasites:										
Filaria										
No. Examined	54		56		48		55		35	
No. with Wuchereria bancrofti	3	5.6	4	7.1	9	18.8	6	10.9	4	11.4
Malaria										
No. Examined	64		67		53		60		50	
No. with parasites	0	0.0	0	0.0	0	0.0	0	0.0	6	12.0
Plasmodium vivax	0	0.0	0	0.0	0	0.0	0	0.0	6	12.0
Trophozoite unidentified	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

	Total	
	No.	%
Blood Parasites:		
Filaria		
No. Examined	1,262	
No. with W. bancrofti	121	9.6
Malaria		
No. Examined	1,406	
No. with parasites	68	4.8
Plasmodium vivax	66	4.7
Trophozoite unidentified	2	0.14

Figure 14

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Summary: Approximately 92 per cent of 2145 Okinawans were parasitized, 90 per cent having worms and 41 per cent protozoa. Hookworm was the dominant parasite. It is believed that the development of the infective larvae of this parasite as well as of Strongyloides stercoralis is fostered by the raising of sweet potatoes. The incidence was 72 and 13 per cent respectively. The parasite density index for hookworm was higher than in most areas of Japan and Korea. The incidence of 49 per cent for Ascaris was considerably less than reported in Japan. However, whenever it was present the infection was heavy as indicated by a parasite density index of 172. The incidence of whipworm was low (21 per cent) and the parasite density suggests that this parasite is not of great importance on Okinawa. Endamoeba histolytica with 13.6 per cent represents one of the highest incidences encountered in our surveys. Malaria was found in 4.8 per cent of the population. Physical examinations suggest that this figure is actually low. However it represents a marked increase over the 0.4 per cent found in 1945. The northern half of Okinawa harbors more malaria than the southern half. This may be due in part to the efforts to control malaria being made by the military forces. Filariasis is endemic on Okinawa and was found in all communities that were surveyed. There was no evidence found to indicate that schistosomiasis was endemic. A few cases of both clonorchiasis and paragonimiasis was encountered.

DETERMINATION OF A STOLL DILUTION EGG COUNT EQUIVALENT FOR EGG COUNTS OBTAINED BY USE OF THE AMS III AND MGL TECHNIQUE: In parasite survey work, an evaluation of the "parasite density" or helminth burden is tantamount in importance to recognition of incidence. The Stoll Dilution Egg Count Technique, commonly used for this determination is routinely impractical for surveys such as the above. Throughout these parasite surveys, numerical indexes have been computed in the manner of Hunter (24) which give comparative indications of the parasite burdens for groups of examinees. Careful egg and cyst counts are made for each coverslip preparation for each parasite involved. Resulting counts are not associated with a unit quantity (gm. or ml.) of feces. Several egg count categories have been established and given "plus" values, see Table XXVII. For any group surveyed a "density factor" is computed by multiplying the number of cases in each plus category by the corresponding index number (the average of the numerical range for the category). The sum of these products is divided by the total positive cases, and the quotient becomes the "density factor". Actually, this figure is the average number of eggs per coverslip preparation for the entire survey group.

Table XXVII. Dilution Egg Count Equivalents for "Plus" Egg Count Categories Determined by AMS III and MGL Techniques

	Egg Count Categories by Concentration Techniques						
	t/	+/	2+/	3+/	4+/	5+/	Total Cases
Ascaris Cases	8	31	26	31	31	48	175
Eggs / ml. (Av. all cases)	1,206	9,358	16,926	27,715	32,731	44,295	
Probable No. of worms	1	7	16	22	26	35	
Hookworm Cases	22	42	32	26	14	8	144
Eggs / ml. (Av. all cases)	304	475	2,554	2,705	8,073	8,745	
Whipworm cases	15	35	30	31	24	29	164
Eggs / ml. (Av. all cases)	216	560	766	1,418	1,232	3,697	

It appeared desirable to convert the egg count categories for our concentration techniques into a Dilution Count equivalent, as of the method of Hood (25). Initially, a series of three-day stool specimens were collected to determine daily fecal output and correction factors for stool consistency and age. Then a parallel series of examinations were run on stools containing variable concentrations of Ascaris, hookworm and whipworm eggs. "Plus" egg count categories were determined by our concentration technique, and Dilution Egg Counts were made.

Of 89 three-day stool specimens which were obtained, 26 were formed, 31 soft formed, and 32 were mushy. Correspondingly, the average daily weights for all stools in each category were 78, 110, and 168 grams. These figures represent closely the ratio of 1:1, 5:2, obtained by previous investigators, and we have used the correction factors of 1, 1.5 and 2 for formed, soft formed, and mushy, respectively. Limited figures available on children indicated that the above figures may be inadequate for correction of stool consistency in the case of children. The arbitrary figure of 80 grams has been used as the average daily fecal output (formed basis) for the adult Japanese examined. The average daily output, uncorrected, for the entire 89 cases was approximately 120 grams. The cooperation of the examinees was such that it seemed the average obtained represented closely the actual fecal output.

Age correction factors on the basis of stool weights in our investigations are in part in agreement with those obtained by Cort, et al (26). Children of 1-2 years discharge about one fourth as much feces as adults and those 3-4 years one-half as much. In our series, children 5-10 produced about 69 per cent of the adult elimination. The above authors found children 5-14 to have a fecal output equal to adults. The age correction factors which we used are .25, .5, .69, and 1.0 for age groups 1-2, 3-4, 5-10, and over 10, respectively.

In Table XXVIII the average Dilution Egg Count is given for all stools examined in each plus category for Ascaris, whipworm and hookworm. For Ascaris the average increased progressively and uniformly from the lowest to the highest category. It would seem, then, that for Ascaris the plus categories appear to hold significance in relation to worm burden. Although this same correlation holds for whipworm, and hookworm, there is less uniformity of increase from one plus category to the next. All 5/ Ascaris cases averaged about 35 worms (both sexes), a moderate worm burden according to Cort, Otto, and Spindler (27). Some of these cases, of course, harbored considerably more, and others, less than 35 worms. Deviations from the average egg counts for Ascaris are shown by subdividing the cases in each "plus" category on the basis of series of Dilution Egg Count categories; Table XXVIII. Then it can be seen that only four of the total of 175 cases had more than 100,000 egg / ml. -- 80/ worms. Of the total cases, 29 or 16.6 per cent had Dilution Egg Counts of 50,000 or more eggs / ml., and for the other plus categories the corresponding percentages were, in descending order, 19.3, 12.7, 3.8, 3.2, and 0.0. This series of percentages should be usable in determining what portion of the individuals examined in our surveys had heavy Ascaris infections.

The plus categories ±, /, 2/ and 3/ include chiefly cases with low or moderate dilution counts. On the other hand, 4/ and 5/ categories include a considerable number of cases with low egg counts. There has been a tendency, then, for the concentration techniques (AMS III and MGL) to recover eggs in considerable numbers even when the infections are light.

Table XXVIII. Distribution of Ascaris Cases by Plus-Count in Relation to Dilution Egg Count Categories

Eggs per ml. of Feces — Plus Categories	/	1/	2/	3/	4/	Estimated No. Of Worms	Severity Categories of Cort et al.
No. of Cases	8	31	26	31	31	48	
1-2,499	7-87.5%	10-32.3%	2-7.6%	0	0	0	1-2 Light
2,500-14,999	1-12.5%	17-54.9%	13-49.6%	8-25.8%	10-32.2%	9-18.7%	2-12 10 worms
15,000-24,999	0	1-3.2%	6-23.7%	11-35.5%	3-9.7%	7-14.6%	12-20 Moderate
25,000-49,999	0	2-6.4%	4-15.3%	8-25.8%	12-38.8%	15-31.4%	20-40 10-50 worms
50,000-99,999	0	1-3.2%	1-3.8%	3-9.7%	6-19.3%	14-29.1%	40-80 Heavy 50/
100,000/	0	0	0	1-3.2%	0	3-6.2%	80/ worms

Brown and Cort (28) and Cort, et al (26) give 200,000 eggs as the average daily production for the female Ascaris. Mixed with 80 grams of feces, each gram would contain 2500 eggs. The egg count per ml. divided by this number would give the approximate number of females; this number doubled should represent the probable worm count (both male and female;) see Tables XXVII and XXVIII. Since the ratio of Necator americanus and Ancylostoma duodenale

present in the examinees was not known, it is questionable whether worm estimates should be computed on the basis of egg counts, as A. duodenale females have been recognized to produce two or more times as many eggs per individual as N. americanus (29). Also, the daily output of eggs by whipworm females has never been definitely determined (30), so worm estimates have not been made.

COLLECTION AND DISTRIBUTION OF PARASITOLOGICAL MATERIALS: During the calendar year approximately 84,000 snails were collected from various parts of Japan. These were known or were suspected of serving as intermediate hosts of various parasitic worms infecting man. All of the snails were used in experiments performed at this laboratory, Kofu, the Japanese National Institute of Health, or were forwarded through the Army Medical Department Research and Graduate School to various research agencies in the United States. A summary of these snails appears in table XXIX.

About 15,000 ml. of preserved mixed protozoa and helminth ova were collected from survey areas. This material contained all the common protozoa and helminth ova, but is of special value because of the large numbers of ova present. Other specimens collected from patients were also preserved and added to the teaching material of the section as the opportunity arose. Much of this material was sent to the Distributing Center for Parasitological Specimens for teaching and research purposes, AMDR&GS. A strain of Trypansoma gambiense obtained by the Section in 1947 through the Japanese National Institute of Health was maintained during 1949 by animal passage in white mice.

Table XXIX. Collection and Distribution of Specimens

Specimens Received	Items	ml. of Concentrate	Recipients
Snails - O. nosophora	67,182		
Snails - Segmentina	17,098		
Land and freshwater molluscs	150		
Formalized feces with			
Ascaris lumbricoides		650	
Enterobius vermicularis		100	
Trichocephalus trichiuris		300	
Heterophyes sp. and Metagonimus sp.		500	
Metagonimus sp.		750	
Heterophyes sp.		300	
Clonorchis sinensis		150	
Endamoeba histolytica		600	
Giardia lamblia		100	
Paragonimus sp.		400	
Capillaria sp.		50	
Preserved helminth ova and protozoan cysts		11,000	
Concentrated sputum containing Paragonimus sp.		400	
TOTALS	84,430	15,300	
Specimens Shipped and Consigner			
Snails - O. nosophora	600		AMDR&GS
Teaching kit	20	100	28th Sta. Hosp.
Preserved helminth ova and protozoan cysts	40	200	AMDR&GS
Preserved helminth ova and protozoan cysts	8	160	College of Med. Evang. Loma Linda, Calif.
Preserved helminth ova and protozoan cysts	97	26,560	AMDR&GS
Preserved helminth ova and protozoan cysts	10	200	207th Mal. Surv. Det.
Clonorchis adults	12	60	College of Med. Evang.
Clonorchis adults	6	30	207th Mal. Surv. Det.
Schistosoma japonicum adults	6	30	207th Mal. Surv. Det.
Positive Schistosomiasis sera (human)	3	24	AMDR&GS
Stained Protozoan slides	5		207th Mal. Surv. Det.
Stained Protozoan slides	392		AMDR&GS
Land and freshwater molluscs	150		U. S. National Museum
TOTALS	1,349	27,364	

PUBLICATIONS AND MANUSCRIPTS: A total of 18 manuscripts, abstracts or papers have been finished during the calendar year. These are presented alphabetically by senior author:

1. Abbott, R. T. and Hunter, G. W. III. Studies on Potential Snail Hosts of Schistosoma japonicum. I. Notes on the Amnicolid Snails Blanfordia, Tricula, and a New Genus, Fukuia from Japan. Proc. Helm. Soc. of Washington. 16(2): 73-86, 1949.

2. Hunter, G. W. III, Bennett, H.J., Fry, N.H., See, J., and Greene, E. The Control of Schistosomiasis japonica. V. Studies on the Penetration of Various Types of Unimpregnated Uniform Cloth by Cercariae of Schistosoma japonicum. Am. J. Trop. Med. 29(5): 723-737, 1949.

3. Hunter, G.W. III, Dillahunt, J. A., and Dalton, H.C. Ms. - The Epidemiology of Schistosomiasis japonica in the Philippine Islands and Japan. I. Survey for Schistosomiasis japonica on Mindoro, P.I. Am. J. Trop. Med. (In press).

4. Hunter, G.W. III, Shillam, D.S., Trott, O.T., and Howell, E.V. Schistosome Dermatitis in Seattle, Washington, J. Parasit. 35(3): 350-254, 1949.

5. Hunter, G.W. III and Hunter, F.H. College Zoology, 821 pp. W. B. Saunders Co., Philadelphia, Pa., 1949.

6. Hunter, G. W. III and Warren, V.G. Ms. - Studies on Filariasis Vi. Observations of the Reversal of Microfilarial Periodicity in a Case of Filariasis Bancrofti, J. Parasit. (In press).

7. Hunter, G. W. III, Ritchie, L. S., Chang, I.C., Rolph, W. D. Jr., Mason, H.C., and Szewczak, J. T. Parasitological Studies in the Far East. VII. An Epidemiological Survey in Southern Korea. J. Parasit. (Suppl.) (Abstract) 35: 28, 1949.

8. Hunter, G. W. III, Ritchie, L. S., Tigertt, W.D., Lin, S., Pan, C., and Tanabe, H. - Immunologic Studies. I. Experiments with Bird and Human Schistosomes. J. Parasit. (Suppl.) (Abstract) 35:28, 1949.

9. Hunter, G. W. III and Abbott, R. T. Studies on Potential Snail Hosts of Schistosoma japonicum, II. Infection Experiments on Amnicolid Snails of the Genera Blanfordia, Tricula, and Fukuia. Proc. Helm. Soc. of Washington. 16 (2): 86-89, 1949.

10. Ingalls, J. W. Jr., Hunter, G.W. III, McMullen, D. B., and Bauman, P.M. The Molluscan Intermediate Host of Schistosomiasis japonica. I. Observations on the Conditions Governing Hatching of the Eggs of Schistosoma japonicum. J. Parasit. 35 (2): 147-151, 1949.

11. Kaufman, E., Hunter, G. W. III, and Pan, C. Protection Experiments with Copper Oleate Ointment against Schistosomiasis. J. Parasit. (Suppl.) (Abstract) 35:29, 1949.

12. McMullen, D. B. Notes on the Course of a Pinworm Infection. J. Parasit. 35(5): 542-543, 1949.

13. McMullen, D. B., Endo-Itabashi, T., Komiyama, S. Seasonal Studies on Schistosoma japonicum in the Intermediate Host Oncomelania nosophora. J. Parasit. (Suppl.) 35: 28 (Abstract), 1949.

14. Ritchie, L. S., Hunter, G. W. III, Pan, C., Yokogawa, M., Szewczak, J. T. Parasitological Studies in the Far East. VI. An Epidemiological Survey of Kyushu Island, Japan. J. Parasit. Suppl.) (Abstract) 34:41, 1949.

SEROLOGY SECTION

Routine

Exclusive of investigative work 111,399 routine procedures were completed in 1949 (Table I). This constitutes an increase of 76 per cent over the preceding year.

Table I. Routine Serologic Procedures

Kahn* and Cardiolipin Microflocculation Tests	70,803
Kahn* and Cardiolipin Microflocculation Tests (Quantitative)	12,553
Kolmer* and Cardiolipin Wassermann (Serum)	16,382
Kolmer* and Cardiolipin Wassermann (Spinal Fluid)	2,424
Pandy	2,395
Gold Curve	2,406
Blood Grouping	373
Rh_0 Factor Determinations	387
Rh_0 Antibody Titrations	702
Cold Agglutinin Titrations	501
Heterophile Antibody Titrations	2,473
Total	111,399

* During September Kahn and Kolmer antigens were replaced by cardiolipin antigens

As noted in previous Annual Reports, all hospitals in Japan were required to submit sera for testing by this laboratory on any cases shown to be doubtful or positive by the then employed standard Kahn test and Kolmer Wassermann. Despite the desire for Army-wide introduction of procedures utilizing cardiolipin antigen early in the year, material did not become available until September. After conducting at this laboratory two courses of training in the procedures for personnel from hospitals of the entire theater, the cardiolipin microflocculation test, qualitative and quantitative and the cardiolipin Wassermann were instituted as standard procedures. Positive sera are still submitted to allow checking of other laboratories' results. As satisfactory proficiency is demonstrated, the routine duplicate testing is discontinued. Reinstitution of this comparative study will be repeated periodically to maintain a uniform high standard of performance of this basic serologic procedure.

During the first trimester of the year an average of 6,800 tests per month were performed. From then on the average rose to over 10,000 per month. This rise resulted primarily from the assumption by the 406th Medical General Laboratory of all standard tests for syphilis (STS) previously carried out by other laboratories in the Tokyo-Yokohama area.

Research

CARDIOLIPIN EVALUATION STUDIES: When limited amounts of material and equipment were received in February, to effect training of personnel parallel examinations were carried out in a preliminary cardiolipin survey using microflocculation and Wassermann antigens in comparison with Kahn and Kolmer-Wassermann tests (Table II).

With continuing study and preliminary checking of all lots of antigen received for supply to other laboratories it became apparent that occasional lots showed an undesirable degree of diminished sensitivity when tested with selected sera. This observation was brought to the attention of AMDR&GS.

Table II. STS Sensitivity Comparisons: (A) Kahn Test vs Cardiolipin Flocculation Test; (B) Kolmer-Wassermann Test vs Cardiolipin Wassermann Test

Qualitative Test		Sera Examined	Positive No.	Positive %	Doubtful No.	Doubtful %	Negative No.	Negative %	Remarks
A)	Kahn	954	73	7.6	21	2.2	860	90.2	Duplicate random specimens
	CLF	948	87*	9.2	16	1.7	845	89.1	
B)	KW	195	102	52.3	10	5.1	83	42.6	Duplicate selected specimens
	CLW	183	106	57.9	4	2.2	73	39.9	

* 10 sera gave prozone reactions. These were verified by the quantitative cardiolipin microflocculation test.

The quantitative cardiolipin microflocculation test was almost always more sensitive than the quantitative Kahn test.

In general the cardiolipin tests were more sensitive than the conventional tests.

EIGHTH ARMY VENEREAL DISEASE CONTROL PROGRAM: In October, November and December the Surgeon's Office, Eighth Army, requested the 406th Medical General Laboratory to perform STS on specimens from various units in the command. Laboratory data regarding these projects have been consolidated and are presented in Table III.

Table III. STS Survey of Units in Eighth Army Cardiolipin Microflocculation Test

	Positive	Doubtful	Negative	Total Tested
Survey 1	11	2	528	541
Survey 2	1	6	492	499
Survey 3	2	4	278	284
Survey 4*	26	35	613	674
Survey 5	32	31	438	501
Total	72	78	2349	2499
Percent	2.9	3.1	94.0	100.0

* 3 Units combined

It is anticipated that the surveys for venereal disease in Eighth Army Units will continue in 1950. An effort will be made to gain more information concerning the clinical aspects of this program to allow collection of epidemiologic facts.

Rh Studies - While all hospital laboratories in this theater are equipped to perform Rh_0 factor determinations, detection of Rh antibodies is centralized in this laboratory. Continuing efforts at early detection and rise of antibodies in Rh negative women lead to an increased awareness of the danger, and an increasing demand for more numerous and specific tests. By the end of 1949 standard items such as bovine albumen had become available, material had been obtained from AMDR&GS to allow determination of Rh subtypes other than Rh_0, and for performance of the Coomb's test.

DGM: A SOLUTION FOR PRESERVING SHEEP ERYTHROCYTES IN 1% AND 2% SUSPENSIONS: In 1948 it was independently confirmed that whole sheep blood could be successfully stored for prolonged periods in equal volumes of Alsever's solution. Attempts were then made to determine whether washed cells in 2% suspension could be similarly stored. For thin suspensions, Alsever's solution has proved unsatisfactory. Such suspensions were anticomplementary and progressively hemolytic. Other preserving solutions were then sought. Of several tried one appeared promising. It received exhaustive investigation during 1949.

Using a carbohydrate-protein-buffer solution, sensitized 1% and unsensitized 2% sheep cell suspensions were prepared. Simultaneously, cells from the same sheep were similarly prepared in conventional solutions, those being buffered and unbuffered isotonic saline. At intervals erythrocytes from each suspension were examined for changes in their properties. Alterations in fragility, cell count, cell size, pH, hematocrit and other characteristics came under investigation. Concomitantly, Wassermann tests on routinely submitted sera were carried out in duplicate, employing cells which had been experimentally preserved and cells prepared fresh on the day of test. Initial findings indicated that the experimental diluent might prove useful. It appeared that the stored cells tended to become stable (homogenous?) with respect to susceptibility to tonic and immune lysis. Further, it was observed that the new preservative effected greater stability of complement. The duplicate Wassermann tests gave little difference in results regardless of whether the cells suspensions were stored or freshly prepared. These encouraging findings stimulated further examination of the experimental diluent. Accordingly the preservation periods were extended.

In May the investigations of 1% sensitized sheep cells stored in three different solutions brought forth interesting data. Kolmer-saline, Alsever's solution and the experimental preparation (DGM) represented the three solutions studied. The preserved erythrocytes were again examined with respect to their fragility, spontaneous lysis, and mean corpuscular volume (Table IV) and applicability to the Kolmer-Wassermann tests.

Table IV. Properties of Sensitized Sheep Erythrocytes in 1% Suspensions

Pres. Fluid	Days Stored	Hemol. percent	Cell Count millions	Frag. Test; NaCl Sol. giving 50% Hem. percent	Hematocrit, percent	MCV µ3
DGM	1	0.2	3.75	0.75	13.0	34.8
	6	0.15	3.75	0.72	11.7	31.2
	16	0.5	3.74	0.71	11.7	31.3
	33	0.2	3.75	0.70	12.7	33.9
	51	0.7	3.73	0.72	13.0	34.9
Alsever	1	0.75	3.57	0.80	13.1	36.7
	6	0.7	3.57	0.73	12.4	34.8
	16	1.4	3.55	0.62	11.0	31.0
	33	3.9	3.45	0.58	9.1	26.4
	51	10.4	3.21	0.69	9.2	28.7
Kolmer	1	3.9	3.74	0.76	13.3	35.6
	6	9.1	3.54	0.76	12.9	36.5
	16	16.2	3.37	0.77	11.2	33.3
	33	29.0	2.79	0.81	9.3	33.4
	51	55.0	1.82	0.86	5.6	32.4

These studies were extended during the following months. Cells preserved in DGM were used repeatedly in Kolmer-Wassermann tests. Previous observations were broadened. The experimental suspensions were subjected to rigorous test by including serum mixtures designed to give greater numbers of partially positive reactions. For purposes of comparison, tests with routinely prepared cell suspensions were performed in parallel.

At the end of 1949 six different lots of stored cells had been carefully studied. The results have been consolidated for presentation in Tables V and VI. Comparison of data accumulated through May may be made with those through December. Good correspondence of agreement and disagreement totals for the respective periods will be observed.

Table V. Evaluation of Stored Erythrocyte Suspensions. Data Recorded in Terms of Agreement and Disagreement with Freshly Prepared Suspensions. All Sera Examined in Parallel by Kolmer-Wassermann Test.

Preserved Suspensions			Comparison by Category*				Comparison by Degree**			
Weeks Stored	Different Lots Used	Sera Tested	Agreement		Disagreement		Agreement		Disagreement	
			No.	%	No.	%	No.	%	No.	%
9	6	1701x	1671	98.2	30	1.8	1568	92.2	133	7.8
9	3	870xx	854	98.2	16	1.8	810	93.1	60	6.9

Table VI. Comparison of Duplicate Kolmer-Wassermann Tests Using Freshly Prepared Suspensions Only

Sera Tested	Comparison by Category*				Comparison by Degree**			
	Agreement		Disagreement		Agreement		Disagreement	
	No.	%	No.	%	No.	%	No.	%
206x	198	96.1	8	3.9	185	89.8	21	10.2
186xx	179	96.2	7	3.8	169	90.5	17	9.5

* Refers to Negative, Doubtful and Positive Classifications
** Refers to Negative, ±, 1+, 2+, 3+ and 4+ classifications.
x Sera tested through December 1949.
xx Sera tested through May 1949.

Heterophile Agglutination Tests with Preserved Erythrocytes - Two per cent suspensions of sheep red cells were prepared in the DGM solution described earlier. At intervals these preparations were subjected to heterophile agglutination tests using routinely submitted sera. For purposes of comparison duplicate quantities of serum were tested in parallel with freshly prepared cells. These comparisons of stored and fresh erythrocytes continued until a limiting time of preservation could be determined. Tables VII A and VII B summarize the results obtained with four different sheep cell suspensions.

It appeared that sheep erythrocytes stored for at least one month gave satisfactory results. Two points are noteworthy: a) Complete agreement between reactions with stored and fresh suspensions was not consistently achieved. However, a relatively close correspondence was obtained, for in no case did end-point differences exceed more than one dilution. b) That this minor degree of deviation was inconsequential seemed to be substantiated by the frequent similar differences observed when fresh suspensions only were tested in duplicate. It therefore seems reasonable to infer that the disagreement noted above may have resulted from technical and experimental deviations and not from the intrinsic character of the preserved cells. If the criterion of full agreement between stored and fresh cells had been permitted a deviation of one dilution more or less, 100% accord would have been achieved.

When attention was directed to the effect of prolonged storage, it was observed that suspensions preserved more than 6 weeks seemed less agglutinable than fresh cells. Here again, however, the reactivity fell only one dilution. This minor decline in reactivity may possibly constitute an advantage since more conservative diagnoses would thus be favored.

Table VII A. Evaluation of Preserved vs. Freshly Prepared 2% Sheep Erythrocyte Suspensions; Determined by Heterophile Agglutination Titrations Performed in Parallel

Sera Titrated	Time Preserved	Agreement	Disagreement	Endpoint* Difference Preserved Cells Higher by 1 tube	Higher by >1 tube	Lower by 1 tube	Lower by >1 tube
No.	Weeks	No.	No.				
41	1	34	7	4	0	3	0
35	2	31	4	2	0	2	0
27	3	27	0	0	0	0	0
35	4	25	10	4	0	6	0
11	5	9	2	2	0	0	0
50	6	39	11	7	0	4	0
15	7	13	2	0	0	2	0
6	9	1	5	1	0	4	0
6	10	4	2	0	0	2	0
31	12	20	11	0	0	11	0
257		203 (79%)	54 (21%)	20 (7.8%)	0	34 (13.2%	0
		Duplicate Tests with Fresh Suspensions					
				Endpoint Difference from Original Test			
293		248 (84.7%)	45 (15.3%)	20 (6.8%)	0	25 (8.5%)	0

Table VII B. Evaluation of Preserved vs. Freshly Prepared Sheep Erythrocyte Suspensions

2% Cell Suspension	Total Sera Tested**	Neg. Sera	Positive sera giving endpoints in dilution of 1: 7	14	28	56	112	224	448	896	1792	3584	7168	14336
Preserved	257	33	51	57	36	18	16	11	15	9	3	4	3	1
Fresh	257	36	50	49	33	24	20	12	13	10	3	4	2	1
			Duplicate Tests with Fresh Suspensions											
Original	293	36	51	52	44	29	23	13	22	16	2	2	2	1
Duplicate	293	34	48	59	43	28	20	15	21	16	3	2	3	1

* The endpoint represented the highest dilution giving definite macroscopic agglutination. Endpoints for given sera never differed by more than one dilution.

** Sera were tested periodically with fresh suspensions and suspensions preserved over a period of 83 days.

Summary: The studies pursued in 1949 suggested that DGM was valuable for preserving sheep erythrocytes in 1% and 2% suspensions. These suspensions, although stored for more than a month, required no further processing. This advantage was noted not only for heterophile agglutinin determinations but also for Kolmer-Wassermann tests. In the latter case, the stored sensitized cells made amboceptor titrations unnecessary.

ENDPOINT OF 50% HEMOLYSIS: Throughout 1949 the experimental complement fixation test employing the unit of 50% hemolysis was studied. This work culminated in the preparation of a manuscript for publication.

The manuscript's relatively complex text and the vital tables and graphs in it prevent effective condensation. It is therefore reproduced in its original form.

Beginning with the observations of Leschly (1) in 1914 and those of Morse (2, 3) and Brooks (4), evidence was presented which pointed out the desirability of titrating complement to the end-point of 50 per cent hemolysis. Wadsworth, Maltaner, and Maltaner (5, 6, 7, 8) systematically applied their technic to diagnostic tests for syphilis, tuberculosis and gonorrhea. In 1943 Friedewald (9), followed in 1946 by Mayer, Eaton, and Heidelberger (10) and by Kent, Bukantz, and Rein (11), independently showed that electro-colorimetric devices enhanced the accuracy and reproducibility with which the unit of complement[1] could be titrated. However, despite increased validity and precision provided by those technics, the majority of laboratories engaged in complement fixation procedures continue to quantitative complement and antisera using complete hemolysis as a criterion. The present paper will describe a quantitative method of titrating syphilitic sera. Although some technics suggested by other investigators have been adopted in part, the fundamental concepts and essential procedures used herein follow new avenues of approach.

Materials

1. Spectrophotometer. A Coleman Junior Clinical Spectrophotometer, model 6, was employed to determine degree of hemolysis. Kahn tubes of 10 mm inside diameter were standardized by calibration with a given solution of hemolyzed sheep cells.

2. Diluent. All reagents were diluted with veronal-buffered saline, modified by addition of 0.02 per cent Bacto gelatin. Preliminary study indicated that gelatin reduced the deterioration rate of complement and retarded spontaneous lysis of sheep erythrocytes (12). A stock buffer solution was prepared, slightly modified from that recommended by Mayer (13): 42.5 gm NaCl, 2.875 gm. barbital, 1.875 gm Na barbital, 0.083 gm $CaCl_2$ (anhydrous), 0.616 mg $MgSO_4$ ($7H_2O$) and water to make 1000 ml. As required, 0.20 gm of Bacto gelatin was dissolved in 100 ml of boiling distilled water. When cool this was mixed with 200 ml of the stock buffer solution and distilled water was added to 1000 ml.

3. Complement. Lyophilized guinea pig serum reconstituted to its original volume with Green (14) diluent was used. If its activity was low it was reconstituted to a slightly lesser volume.

4. Amboceptor. Anti-sheep erythrocyte hemolysin preserved with an equal volume of glycerol was employed.

5. Sensitized Erythrocytes. Two hundred ml of sheep blood were collected aseptically in a 600 ml transfusion vacuum bottle containing 200 ml of modified Alsever's solution (15, 16). This suspension was stored at 4°C.

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[1] The unit of complement producing 50 per cent hemolysis is hereafter designated K.

A quantity of preserved blood sufficient for one day was removed aseptically and centrifuged. The sedimented cells were washed three times with diluent and from a packed state were made up to about 2.5 per cent suspension. Two-tenths ml of this cell suspension was laked with 1.8 ml of distilled water. The resulting hemoglobin solution was centrifuged and the optical density (OD) of the supernate determined spectrophotometrically at 550 mμ using distilled water as reference zero. From the reading so obtained the additional volume of diluent needed to adjust the cell suspension to an optical density of 0.400 ± 0.01 was calculated from the formula

$$\text{Volume to be added} = V \frac{OD - 0.400}{0.400}$$

where V designates the quantity of sheep cell suspension to be adjusted. The standardized preparation corresponds to a 2 per cent suspension containing 500,000 erythrocytes per mm^3.

One volume of an optimal dilution of amboceptor was added slowly, with continuous stirring to one volume of the adjusted cell suspension. The mixture was kept at room temperature for at least 20 minutes then stored in the refrigerator until needed.

Technic for Titrating Complement

A 1:20 dilution of complement was prepared by adding 0.5 ml of reconstituted complement to 9.5 ml of diluent. From this a 1:200 dilution was made and titrated, as shown in Table VIII.

Table VIII. Titration of Complement

REAGENT (ml.)									REFERENCE* H_{100}			REFERENCE** H_{50}			REFERENCE*** H_0
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Complement, 1:20									.40	.40	.40	.40	.40	.40	
Complement, 1:200	.20	.25	.30	.35	.40	.45	.50	.55							.40
Diluent	1.00	.95	.90	.85	.80	.75	.70	.65	.80	.80	.80	.80	.80	.80	.80
Cells sensitized with optimal amboceptor concentration	.80	.80	.80	.80	.80	.80	.80	.80	.80	.80	.80	.40	.40	.40	.80
Supernate of sensitized cells												.40	.40	.40	

Incubate in 37 C waterbath for 30 minutes then centrifuge at 2000 rpm for 10 minutes. Read optical densities of supernates using H_0 as reference zero.

Typical Results

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Optical density at 550 mμ	.049	.108	.211	.319	.421	.490	.563	.630	.713	.713	.712	.376	.376	.375
Mean optical density										.713		.376		
Per cent hemolysis	6.9	15.2	29.6	44.8	59.1	68.8	79.0	88.5				52.7		

* H_{100}, H_{50} and H_0 refer to 100, 50 and 0 per cent hemolysis respectively.

** This 50 per cent reference set provides OD and per cent hemolysis values corrected for deviation from the Lambert-Beer Law.

*** Mixture of complement and diluent is inactivated at 56 C for 30 minutes.

After incubation and centrifugation, optical densities of the supernates of all tubes were determined at 550 mμ wavelength using tube number 15 as reference zero. The mean optical densities of the three 100 per cent and the three 50 per cent hemolysis reference tubes were obtained. Per cents hemolysis were calculated by applying the relation

$$\text{Per cent hemolysis} = \frac{OD}{OD_{H_{100}}} \times 100$$

where $OD_{H_{100}}$ indicates the mean optical density of the 100 per cent hemolysis reference set and OD represents the optical density of each of the other tubes.

The per cent hemolysis for each titration tube was plotted against its corresponding volume of 1:200 complement on a standard graph form (Fig. 1A) and a straight line fitted to the plotted points.

The construction of the graph form was suggested by earlier workers, conspicuously Morse (3) and Thompson and Maltaner (17) and derives from Von Krogh's alternation equation (18):

$$\text{(I)} \qquad x = K \left(\frac{y}{1-y} \right)^{1/n}$$

or in logarithmic form,

$$\text{(II)} \qquad \log x = \log K + \frac{1}{n} \log \frac{y}{1-y}$$

In these relations x represents the volume of complement used and y the corresponding per cent of hemolysis. A plot of log x against log $\frac{y}{1-y}$ results in a straight line. Constants, 1/n and K, respectively, denote the slope of the hemolytic curve, about 0.200, and the quantity of complement giving 50 per cent hemolysis (one unit).

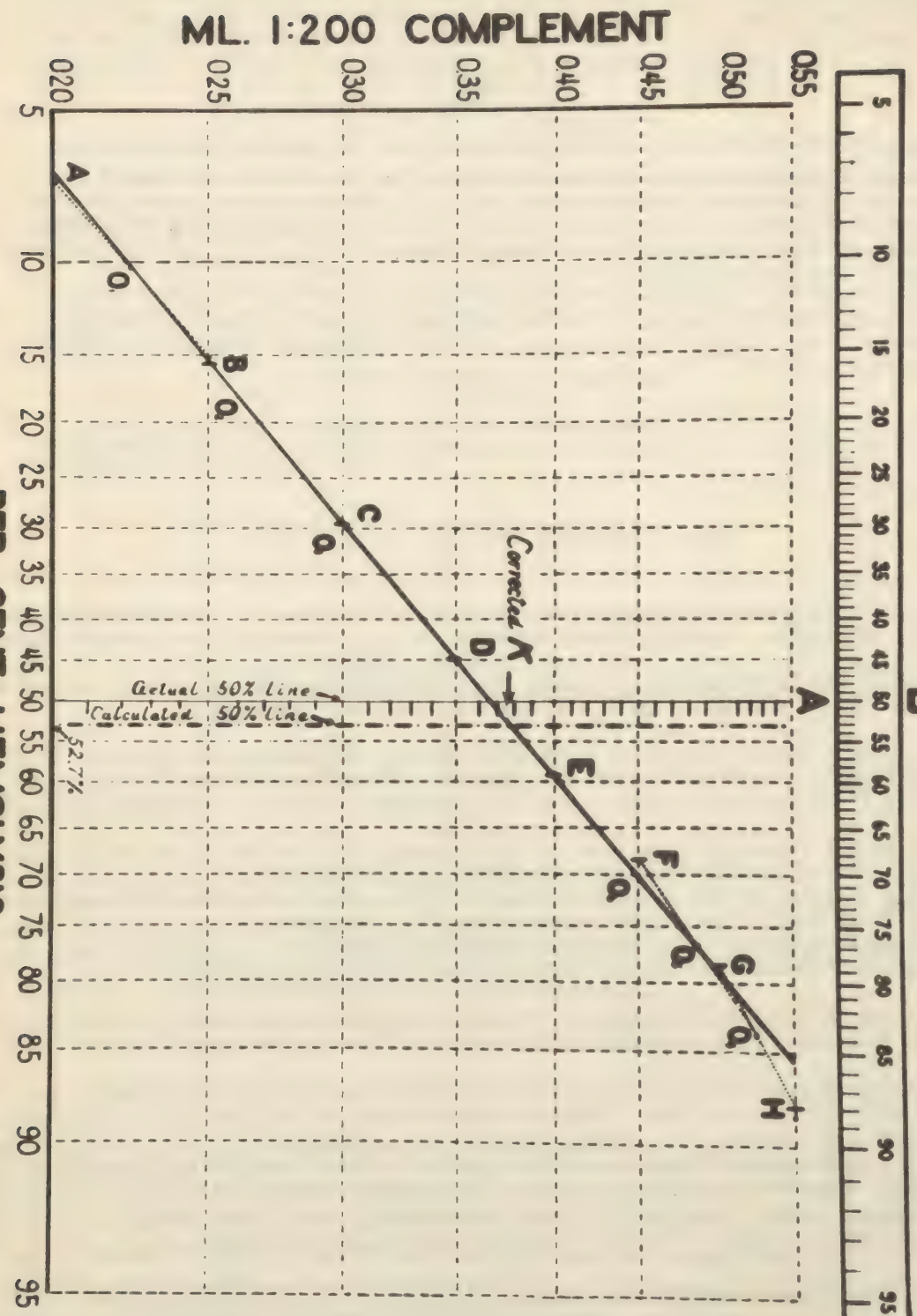
The ordinate values shown in the standard graph form indicate the amount of 1:200 complement routinely used in titrations. They are spaced logarithmically and are set off by employing the C scale of a slide rule. The abscissa graduations represent log $\frac{y}{1-y}$ for the different values of y and are set off by using the B scale of the slide rule. First, a horizontal line is drawn and an origin representing 50 per cent ($y = 0.50$) assigned. The distance on the slide rule from 5 to 95 is indicated left of the origin and marked 5 per cent. The distance from 10 to 90 is set off and marked 10 per cent. The other percentage points are similarly designated until the origin is reached. Percentages greater than 50 are set off right of the origin in like manner.

To interpolate points intermediate to those indicated on the graph, a rule is calibrated in increments of one throughout the range of 5 to 95 per cent (Fig. 1B).

To eliminate subjectivity in fitting a straight line to the plotted points the following procedure was adopted: Using a straight edge the midpoint O_1, between A and B was marked; the midpoint, O_2, between O_1 and C was marked, and similarly other midpoints were set off progressively until that coordinate on or just left of the 50 per cent line was reached. Likewise, midpoints from H to E were progressively determined. Using O_3 and O_6 as guide points a straight line was drawn through them. This method also tended to give appropriate weight to values closest to the 50 per cent point.

A perpendicular was projected from the calculated value of the H_{50} reference set (52.7 per cent for the conditions shown in Fig. 1A). That point on the ordinate where the perpendicular intersects the plotted line gives corrected K in ml of complement diluted 1:200, or K/200 ml of undiluted complement.

Figure 1A and 1B



For complement fixation tests and antigen titrations both 1K and 2K in 0.4 ml volumes were used. A dilution containing 2K in 0.4 ml should theoretically be obtained by mixing K ml of undiluted complement (200 units) with sufficient diluent to make 40 ml total volume. However, as observed by other investigators, actual experience revealed that approximately 10 per cent deterioration of complement resulted from overnight refrigeration. To compensate for this loss, in practice K ml of undiluted complement (200 units) was carefully pipetted into a suitable graduated cylinder and diluent was added to make 36 ml only. Fifteen ml of this mixed with 15 ml of diluent provided 30 ml of dilution calculated to contain 1K in 0.4 ml.

Titration of Amboceptor

Each unused lot of glycerolized amboceptor to be titrated was inactivated at 56 C for 30 minutes. For titrations a 1:500 dilution was made by mixing 0.2 ml of amboceptor with 49.8 ml of diluent. Further dilutions were prepared (19), then each was employed to sensitize an equal volume of sheep erythrocytes as shown in Table IX.

Table IX. Preparation of Amboceptor Dilutions and Sensitized Cells

	DILUTIONS 1:						
	500	666	1000	1333	2000	2666	4000
Diluent (ml)	0	0.4	0.8	1.0	1.2	1.3	1.4
Amboceptor 1:500 (ml)	4.0	1.2	0.8	0.6	0.4	0.3	0.2
2 per cent sheep cell suspension (ml)	4.0	1.6	1.6	1.6	1.6	1.6	1.6

The amboceptor titration was carried out as indicated in Table X, the reagents being added in the order given. All tubes were incubated at 37 C for 30 minutes and centrifuged. Their optical densities were determined and the corresponding percentages of hemolysis calculated as for the complement titration.

The per cent of hemolysis for each dilution of amboceptor was plotted on the standard graph form against the two quantities of complement used (0.4 ml and 0.3 ml), (Fig. 2A). The amount of complement necessary to give 50 per cent hemolysis (K) for each dilution of amboceptor was determined from the graph as described in the section on complement titration. The dilutions of amboceptor used were plotted as suggested by Kent (19) on arithmetic paper against the amount of complement necessary for 50 per cent hemolysis. A smooth curve was fitted to the plotted points. The optimal dilution of amboceptor was considered the lowest concentration of amboceptor falling on the apparently linear portion of the curve (Fig. 2B).

It is apparent from Table X and Figure 2A that the value of 1/n is minimum at the optimal dilution of amboceptor. This observation is in conformity with that reported by Kent (20). However, routine amboceptor titrations did not consistently demonstrate this relationship.

Titration of Antigen

A positive luetic serum containing 32 to 64 Kolmer-Wassermann units (500 to 1000 antibody units by 50 per cent titration) was inactivated in the 56 C water bath for 30 minutes. Dilutions of serum were then prepared as summarized below:

Serum Dilution 1:	100	150	200	300	500	800
Diluent (ml)	29.7	2.5	4.0	6.0	8.0	7.0
Undiluted positive serum (ml)	0.3					
Positive serum 1:100 (ml)		5.0	4.0	3.0	2.0	1.0

Figure 2A

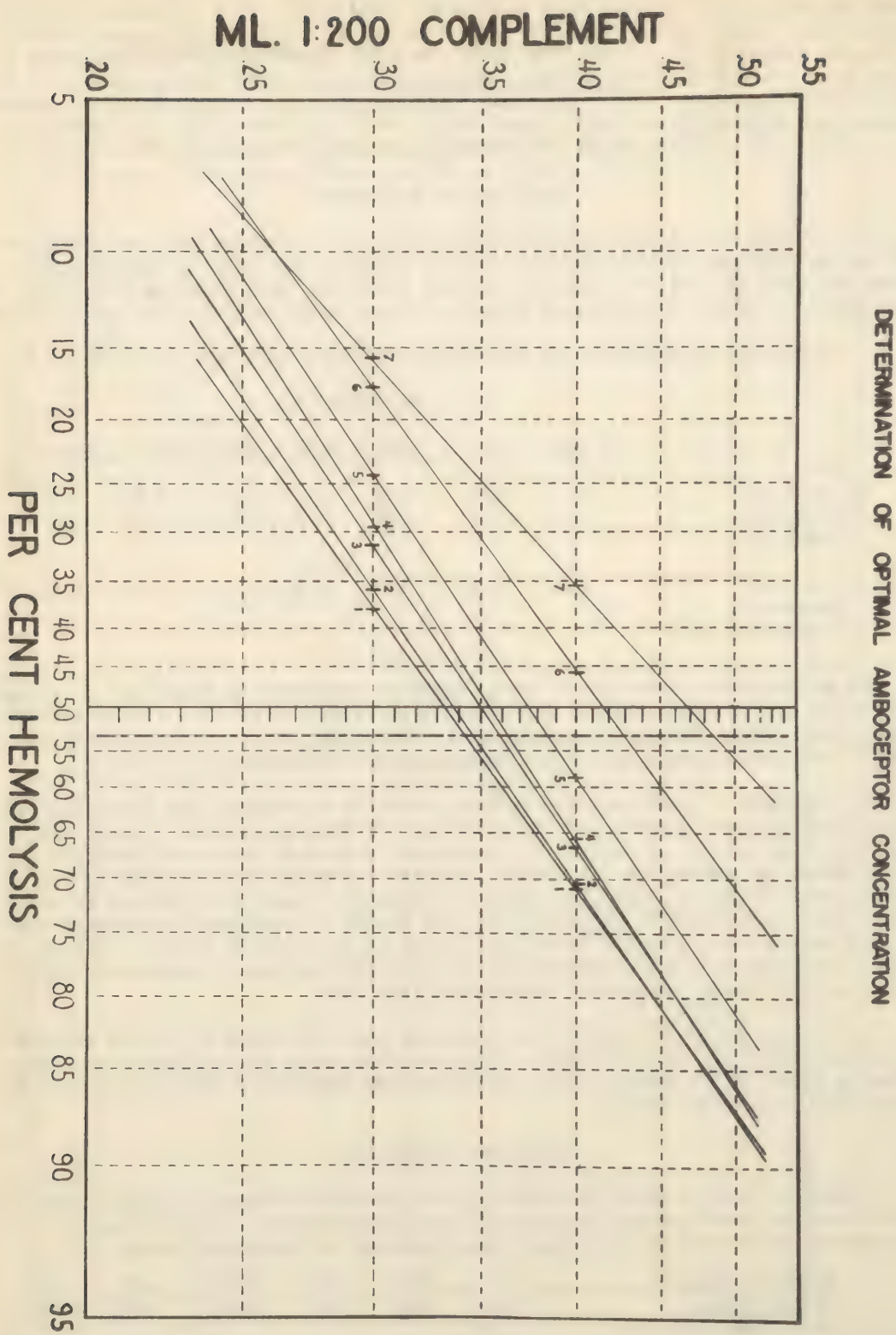

DETERMINATION OF OPTIMAL AMBOCEPTOR CONCENTRATION
ML. 1:200 COMPLEMENT
20 25 30 35 40 45 50 55
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
PER CENT HEMOLYSIS

Figure 2B

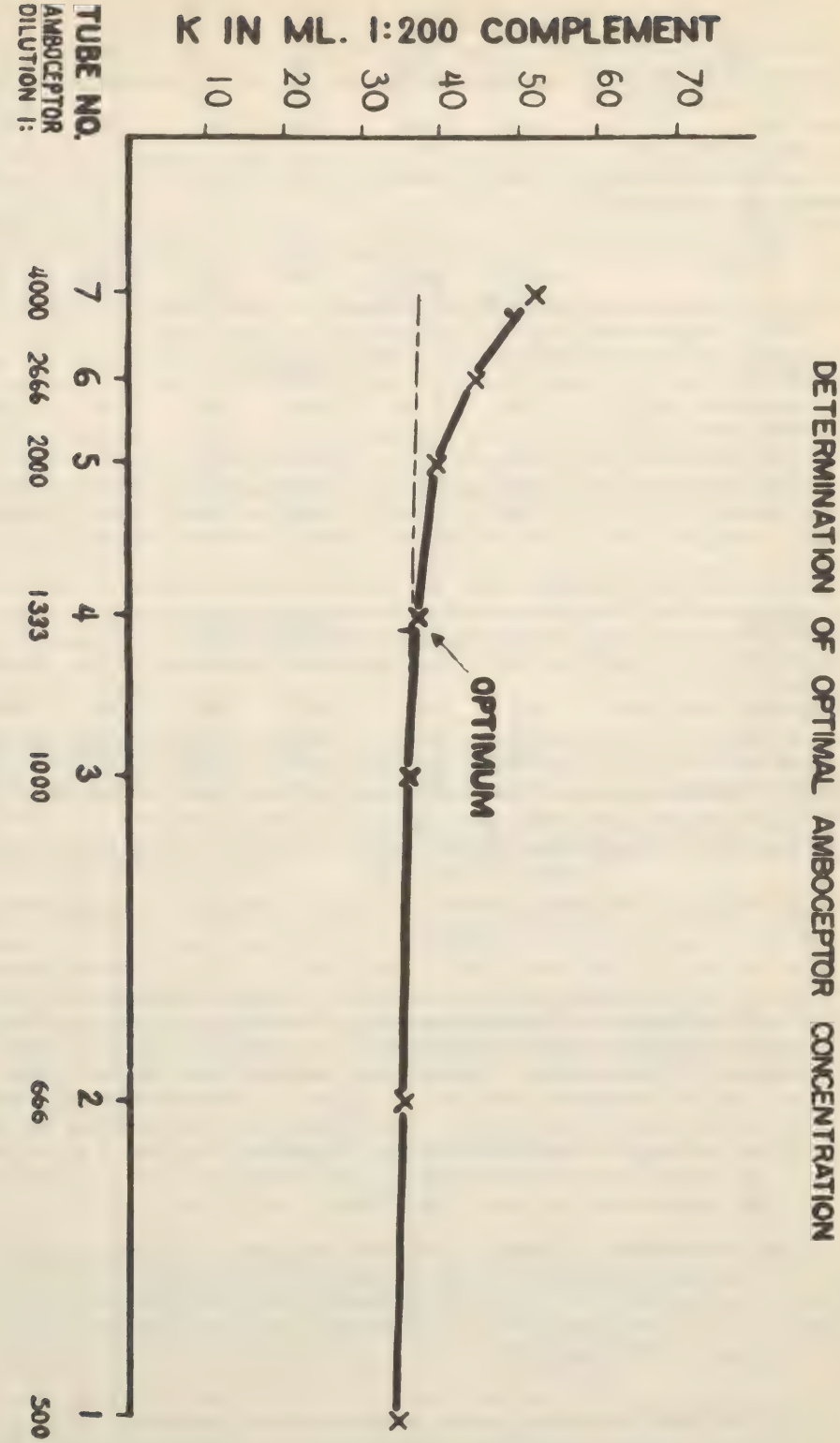

DETERMINATION OF OPTIMAL AMBOCEPTOR CONCENTRATION
K IN ML. 1:200 COMPLEMENT
10
20
30
40
50
60
70
OPTIMUM
TUBE NO.
AMBOCEPTOR DILUTION 1:
7
4000
6
2666
5
2000
4
1333
3
1000
2
666
1
500

Table X. Titration of Amboceptor

TUBE	1	2	3	4	5	6	7
REFERENCE		H_{100}					
1:20 complement (ml)	0.40	0.40	0.40	0.40	0.40	0.40	
1:600 complement (ml)							1.20*
Diluent (ml)	0.80	0.80	0.80	0.80	0.80	0.80	
Cells sensitized with 1:500 amboceptor (ml)	0.80	0.80	0.80	0.40	0.40	0.40	0.80
Supernate of sensitized cells (ml)				0.40	0.40	0.40	
Optical density	0.698	0.697	0.693	0.371	0.360	0.370	
	Mean	0.696		Mean	0.367:	52.7%	

TITRATION	REAR ROW						
1:600 complement (ml)**	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Cells sensitized with amboceptor dilution 1:	500	666	1000	1333	2000	2666	4000
(ml)	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Optical density	0.492	0.490	0.460	0.455	0.407	0.381	0.245
Per cent hemolysis	70.7	70.4	66.1	65.4	58.6	45.7	35.2

TITRATION	FRONT ROW						
1:800 complement (ml) ***	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Cells sensitized with amboceptor dilutionl:	500	666	1000	1333	2000	2666	4000
(ml)	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Optical density	0.265	0.250	0.218	0.208	0.168	0.122	0.109
Per cent hemolysis	38.1	35.9	31.3	29.9	24.2	17.5	15.7

* Inactivated at 56 C for 30 minutes.

** Quantity of complement equivalent to 0.40 ml of 1:200 complement plus 0.80 ml of diluent.

*** Quantity of complement equivalent to 0.30 ml of 1:200 complement plus 0.90 ml of diluent.

Fifteen antigen dilutions were made in 1.5-fold increments as follows: Twelve ml of a 1:100 dilution of Kolmer-Wassermann or cardiolipin antigen were introduced into a small Erlanmeyer flask. From this 4 ml were withdrawn and put into the first of a series of fifteen tubes. The antigen removed from the flask was replaced by 4 ml of diluent and the contents mixed well. Again, 4 ml were withdrawn then pipetted into the second tube of the series. Replacement with diluent and withdrawal of antigen continued similarly until all fifteen tubes had received 4 ml of serially diluted antigen. To obtain greater stability of the antigen in these dilutions, they were kept at room temperature at least three hours before use.*

Eight rows of fifteen tubes each were arranged. All rows through the sixth received 0.4 ml each of serum dilutions 1:100 to 1:800 respectively and 0.4 ml of complement containing 2K. All tubes of the seventh and eighth rows took 0.4 ml of diluent and 0.4 ml of complement 2K. All tubes of the seventh and eighth rows took 0.4 ml of complement containing 1K.

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* Because antigen is one of the reagents added last in the complement fixation test, this three hour ageing period imposes no practical difficulty.

The first tube of each row received 0.4 ml of the first antigen dilution. The second tube of each row took 0.4 ml of the second dilution and in like manner the remaining tubes received corresponding antigen dilutions.

A complement deterioration set was included according to the pattern of the complement titration illustrated in Table VIII. However, here the adjusted dilution containing one complement unit (K) in 0.4 ml. was used instead of the usual 1:200 dilution.

All tubes were shaken well and stored at 4°C for fifteen to eighteen hours. Then 0.8 ml of optimally sensitized sheep cells was added to all tubes of the first seven rows and to all except the fifteenth tube of the complement deterioration set. After mixing, the tubes were incubated in the water bath at 37°C for 30 minutes and centrifuged for 10 minutes at 2000 rpm.

Following overnight refrigeration, however, each tube in the eighth row and the fifteenth tube of the complement deterioration set were inactivated at 56°C for 30 minutes. Then they also received 0.80 ml of sensitized cells and were centrifuged.

The optical densities of all tubes of the antigen titration set were read with the spectrophotometer at 550 mμ wave length using their corresponding tubes of row eight as zero reference. The per cents of hemolysis were then calculated by employing the mean optical density of the H_{100} reference tubes of the complement deterioration set. Table XI summarizes details of the antigen titration.

The quantity K', of complement giving 50 per cent hemolysis in the complement deterioration set was determined in the same manner as described in the section on complement titration. The expression 0.40:K' represents the fraction of one complement unit remaining after overnight refrigeration. The antigen titration was not considered valid if this value was less than 0.8.

Assigning per cents hemolysis to the ordinate and antigen dilutions to the abscissa, the values for each tube in rows one through seven were plotted as shown in Fig. 3. The seventh row indicated the anticomplementary activity of each antigen dilution. If, at the dilution of antigen optimal for complement fixation, the degree of hemolysis read from curve VII was less than 25 per cent, the antigen was regarded excessively anticomplementary and was discarded.

The optimal dilution of antigen is construed as that giving minimal hemolysis in the neighborhood of the 50 per cent zone. From Fig. 3, it can be seen that the optimum lies between points A and B, which show minimal hemolysis of the serum dilutions immediately above and below the 50 per cent zone. This optimum can be determined more exactly by performing complement fixation tests (described in the following section) with the same serum, using dilutions of antigen between A and B. The optimal dilution of antigen is indicated by that intermediate dilution with which the greatest number of antibody units is demonstrated.

The Complement Fixation Test

Technic

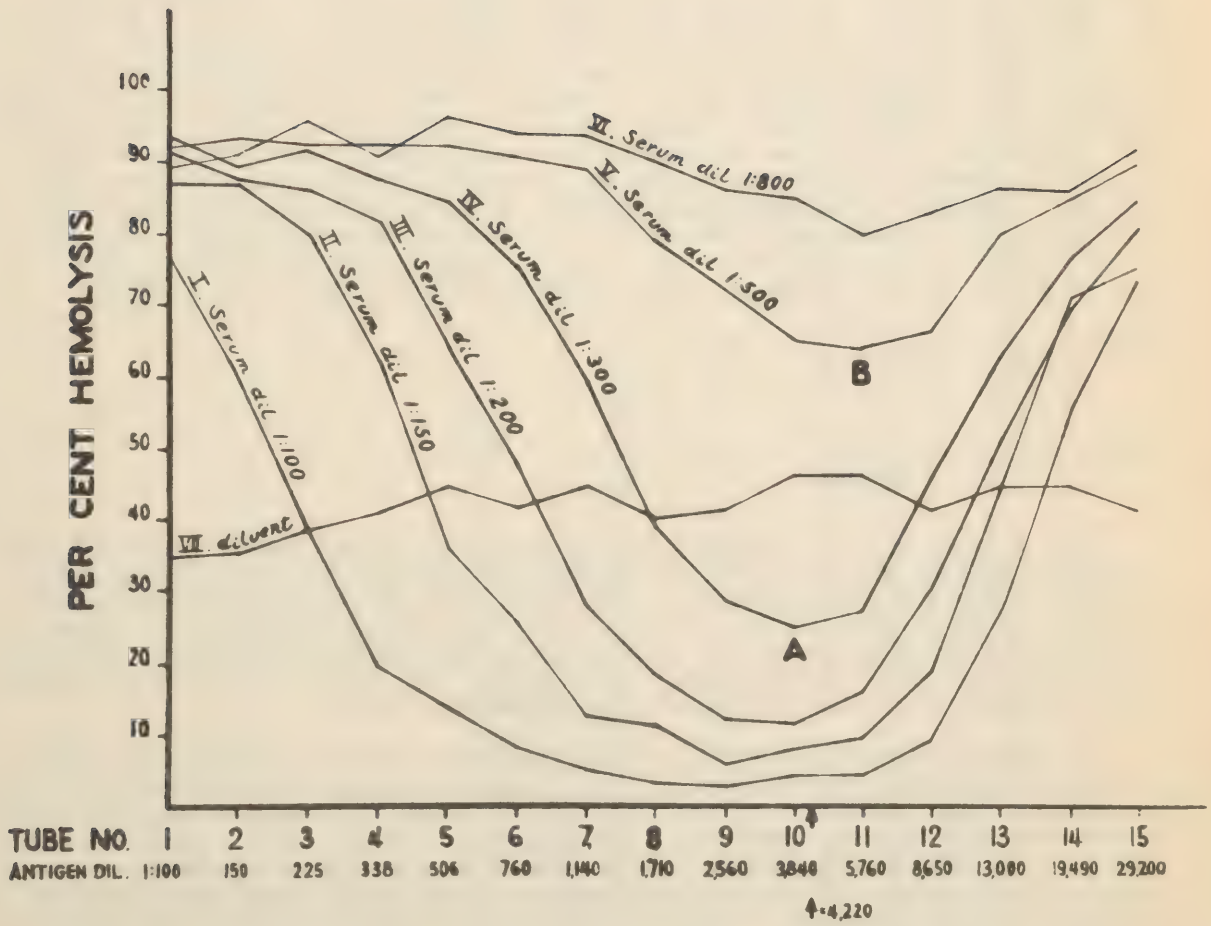
The serum to be quantitated was inactivated in the 56°C water bath for thirty minutes. Two rows of ten tubes each were arranged in a Wassermann rack. The first and the succeeding nine tubes of the front row received 1.2 ml and 0.8 ml of diluent respectively. Four-tenths ml of serum was added to the first tube of the front row and the contents were mixed;of this mixture 0.4 ml was transferred to the first tube of the rear row and 0.8 ml to the second tube of the front row. From this tube 0.4 ml was transferred to the second tube in the rear row and 0.8 ml to the third tube of the front row. The remaining tubes received serum dilutions similarly prepared and distributed. The excess 0.8 ml in the tenth tube of the front row was discarded or held for further dilution.

Table XI. Titration of Antigen

	TUBE		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Antigen Dilution 1: (0.4 ml)		100	150	225	338	506	760	1,140	1,710	2,560	3,840	5,760	8,650	13,000	19,490	29,220
2 Complement Units, 2K (0.4 ml)	I Serum dil. 1:100 (0.4 ml)	OD	0.550	0.442	0.282	0.127	0.097	0.057	0.042	0.022	0.020	0.025	0.025	0.063	0.188	0.406	0.528
		%H	75.2	60.5	38.6	17.4	13.3	7.8	5.1	3.0	2.7	3.4	3.4	1.6	25.7	55.6	76.2
	II Serum dil. 1:150 (0.4 ml)	OD	0.638	0.635	0.578	0.449	0.253	0.188	0.086	0.079	0.038	0.065	0.074	0.143	0.310	0.508	0.560
		%H	87.2	86.8	79.2	61.6	34.6	25.7	11.8	10.8	5.2	8.9	10.1	19.6	42.4	69.6	76.6
	III Serum dil. 1:200 (0.4 ml)	OD	0.665	0.635	0.628	0.580	0.445	0.335	0.200	0.136	0.085	0.085	0.111	0.218	0.363	0.506	0.580
		%H	89.6	86.8	86.0	79.4	61.0	45.8	27.4	18.6	11.6	11.6	15.2	29.8	49.7	69.2	79.4
	IV Serum dil. 1:300 (0.4 ml)	OD	0.670	0.650	0.668	0.640	0.612	0.545	0.431	0.281	0.208	0.180	0.200	0.335	0.450	0.552	0.615
		%H	91.7	89.0	91.4	87.6	83.8	74.6	59.0	38.5	28.5	24.6	27.4	45.8	61.6	75.6	84.2
	V Serum dil. 1:500 (0.4 ml)	OD	0.668	0.670	0.675	0.670	0.675	0.665	0.665	0.573	0.525	0.472	0.465	0.495	0.580	0.610	0.640
		%H	91.4	91.8	92.4	91.8	92.4	91.0	89.6	78.4	71.8	64.6	63.6	67.8	79.4	83.5	87.6
	VI Serum dil. 1:800 (0.4 ml)	OD	0.650	0.655	0.690	0.662	0.700	0.685	0.680	0.656	0.630	0.630	0.585	0.600	0.615	0.620	0.663
		%H	89.0	89.6	94.4	90.8	95.8	93.8	83.0	89.8	86.2	86.2	80.0	82.2	84.2	84.8	90.4
1 Comp. Unit, 1K (0.4 ml)	VII Diluent (0.4 ml)	OD	0.245	0.245	0.268	0.285	0.310	0.290	0.320	0.285	0.300	0.324	0.325	0.297	0.275	0.308	0.297
		%H	33.5	33.5	36.7	39.0	42.4	39.7	43.8	39.0	41.0	44.3	44.5	40.6	37.6	42.2	40.6
	VIII Diluent (0.4 ml)		Store at 4 C overnight, inactivate at 56 C for 30 minutes then, as for the first seven rows, add 0.8 ml of sensitized cells. This row serves as reference zero.														

Figure 3

TITRATION OF ANTIGEN. OPTIMAL DILUTION IS 1 : 4220



The complement fixation test was completed as shown in Table XII. For every ten sera tested a complement deterioration test was included as noted in the section on antigen titration.

Determination of Antibody Titer

In this study K is considered that amount of complement which, in the presence of 0.8 ml of optimally sensitized suspension, lyses half of the erythrocytes. Expressed otherwise it is a unit designating 50 per cent hemolysis. Likewise, the unit of antibody is regarded as that amount which, in the presence of an optimal concentration of antigen and 2K, will fix 1K, leaving 1K free. The unfixed complement theoretically should effect 50 per cent hemolysis. However, the activity may be altered somewhat by the anticomplementary or procomplementary content of the serum.

With these considerations in mind a formula for obtaining the antibody units can be developed from the relation shown in Fig. 4.

Curve GH shows the trend of hemolysis resulting from the activity of one complement unit, (K), in the presence of different serum dilutions. The rising slope, GB, denotes increasing anticomplementary action while the relatively level BH shows that this property has been diluted out leaving full activity of K unimpaired. Curve EF illustrates the trend of hemolysis effected by 2K when mixed with the same serum dilutions and an optimal concentration of antigen. The first four tubes of the series contain serum rich in antibody. Hence, all of the complement is fixed and no hemolysis is observed. However, an antibody dilution progresses, fixation diminished and hemolysis increased. At O, where curve EF crosses GH, 1K has been fixed since the hemolytic activity of the remaining complement coincides with that of K in the control row. The corresponding point, P, on the abscissa therefore indicates the amount of serum containing one antibody unit. The proportionate distance of this point above the next lower dilution of serum is established according to the relations

$$(III) \qquad \overline{MP} = \frac{\overline{AC}}{\overline{AC} + \overline{BD}} \times \overline{MH}$$

$$(IV) \text{ or, } \log p = \frac{\overline{AC}}{\overline{AC} + \overline{BD}} \times \log 2$$

Fig. 4 shows that $\overline{AC}$ represents the difference in optical densities (or per cents hemolysis) between rear and front row supernates at the dilution just preceding that at which 1K has been fixed (point O), while BD denotes the difference between front and rear row supernates of the next higher dilution. These differences in optical density can be read directly with the spectrophotometer at 550 mμ by assigning the rear tube as reference O in the first pair and the front tube as reference O in the second pair. No other pairs need be examined spectrophotometrically.

In routine complement fixation tests the practice has been to obtain the titer of a given serum simply as follows: If all tubes of the rear row show more lysis than the corresponding tubes of the front row, the test serum is considered negative and is reported as containing less than 10 antibody units per ml. If the first tube of the rear row indicates no hemolysis or less hemolysis than the corresponding front tube, succeeding pairs are examined visually until that pair showing more hemolysis in the rear tube is reached. Using the spectrophotometer the optical densities of this and the preceding pair are taken as indicated in the paragraph above. The proportional factor is given by the slightly modified expression for formula (IV):

$$(V) \qquad p = \text{antilog} \frac{OD_{LD}}{OD_{LD} + OD_{HD}} \times 0.301$$

Table XII. Complement Fixation Test

TUBE			1	2	3	4	5	6	7	8	9	10
SERUM DILUTION 1:			4	8	16	32	64	128	256	512	1024	2048
TITER, Antibody Units /ml			10	20	40	80	160	320	640	1280	2560	5120
	Serum, 0.4 ml	Optical **										
	complement, 2K, 0.4 ml	Density	0.010	0.001	0.001	0.002	0.007	0.040	0.548	0.632	0.648	0.645
Rear	Antigen, 0.4 ml	Per Jent **										
Row	Sensitized cells, 0.8 ml*	Hemolysis	1.4	0.2	0.2	0.3	1.0	5.6	77.0	88.7	91.0	90.6
	Serum, 0.4 ml	Optical **										
Front	Complement, 1K, 0.4 ml	Density	0.048	0.088	0.172	0.250	0.276	0.311	0.331	0.342	0.358	0.348
Row	Diluent, 0.4 ml	Per cent **										
	Sensitized cells, 0.8 ml*	Hemolysis	6.7	12.4	24.2	35.1	28.8	43.6	46.5	48.1	47.5	48.9

Optical density of front tube using corresponding rear tube as reference zero: 0.271***

Optical density of rear tube using corresponding front tube as reference zero: 0.217***

Calculation of proportionate distance and antibody units per ml:

$$\log p = \frac{0.271}{0.271 + 0.217} \times 0.301 \; ; \; p = 1.47$$

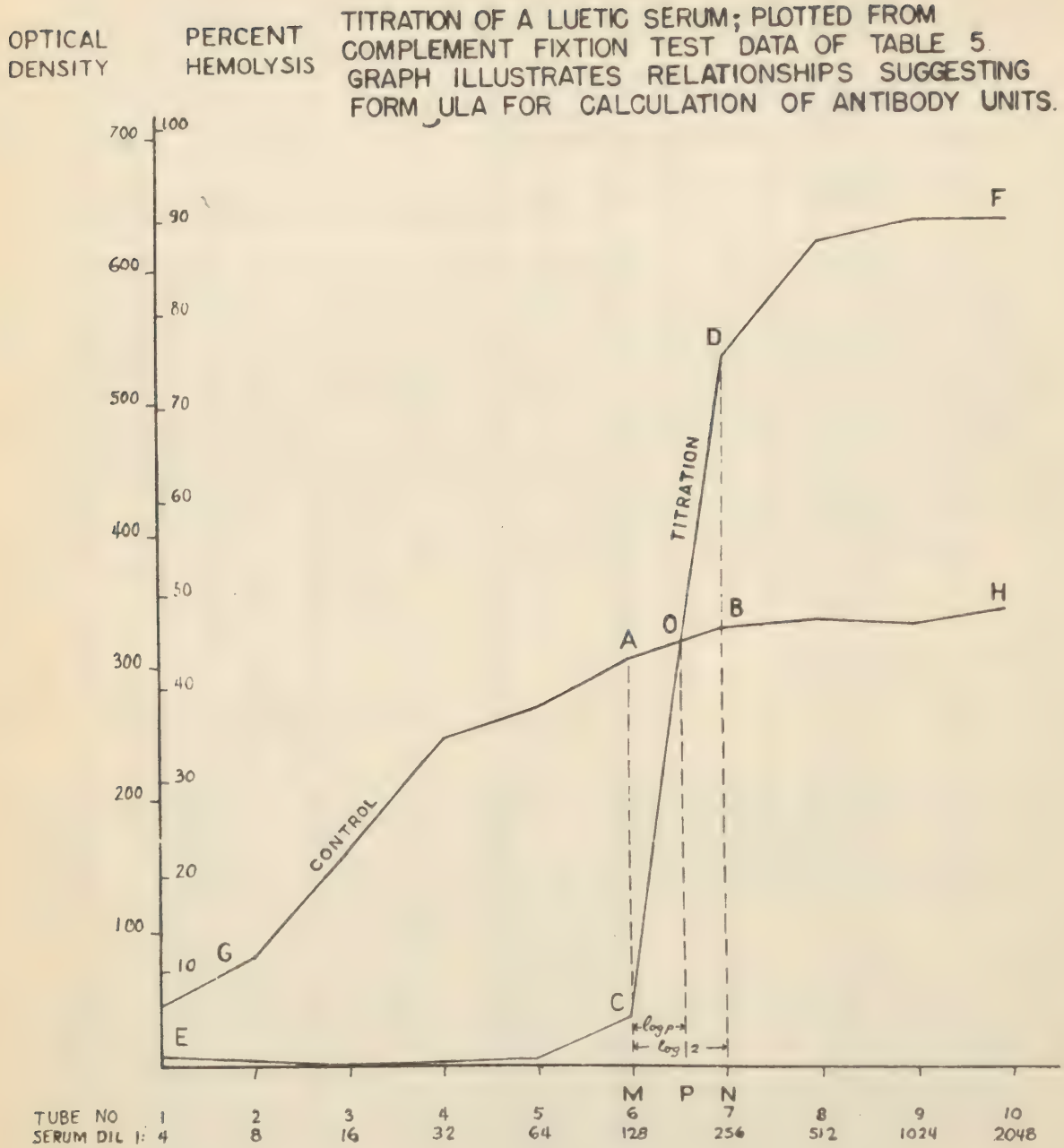
$T_{LD} = 320$; $T_{LD} \times p$ $\quad 320 \times 1.47 = 470$ antibody units /ml

* Added to immune system which was refrigerated at 4 C overnight. Mixture then incubated at 37 C for 30 minutes and centrifuged at 2000 rpm for 10 minutes.

** Optical densities read and per cents hemolysis calculated usi ng reference H_{100} and H_0 of the complement deterioration set. These determinations are recorded merely to provide data for plotting the curves of Fig. 4.

*** In routine complement fixation tests only these two readings are necessary for computation of the antibody titer.

Figure 4


OPTICAL DENSITY
PERCENT HEMOLYSIS
TITRATION OF A LUETIC SERUM; PLOTTED FROM COMPLEMENT FIXTION TEST DATA OF TABLE 5. GRAPH ILLUSTRATES RELATIONSHIPS SUGGESTING FORMULA FOR CALCULATION OF ANTIBODY UNITS.
700
600
500
400
300
200
100
100
90
80
70
60
50
40
30
20
10
F
D
TITRATION
H
B
A
O
CONTROL
G
C
E
log p
log 2
M
P
N
TUBE NO 1 2 3 4 5 6 7 8 9 10
SERUM DIL 1: 4 8 16 32 64 128 256 512 1024 2048

where OD_{LD} and OD_{HD} signify optical densities of the low and high dilutions and p represents the proportional factor.

To avoid calculation, the proportional factor can be determined by means of the nomogram shown in Fig. 5. The horizontal arms representing optical densities of the low and high dilutions respectively are constructed with a centimeter rule, while the vertical arm is set off with the C scale of a slide rule. A straight line joining the two critical optical densities will cross the vertical scale at a point which indicates the proportional factor.

The relation

$$\text{(VI)} \qquad \text{titer} = \frac{\text{reciprocal of LD} \times p}{0.4}$$

therefore expresses the antibody content of test serum in units per ml.

Application

Sensitivity Studies

Sera from syphilitic patients were titrated simultaneously by the quantitative Kolmer-Wassermann test (33) and by the 50 per cent method described above. The results given in Table XIII are expressed in antibody units and are representative of numerous tests. They show that the 50 per cent procedure measures the antibody content of different sera with marked precision, whereas the Kolmer-Wassermann test does so in the usual categorical unitages which are notably broad in range. Similar findings were observed with spinal fluid.

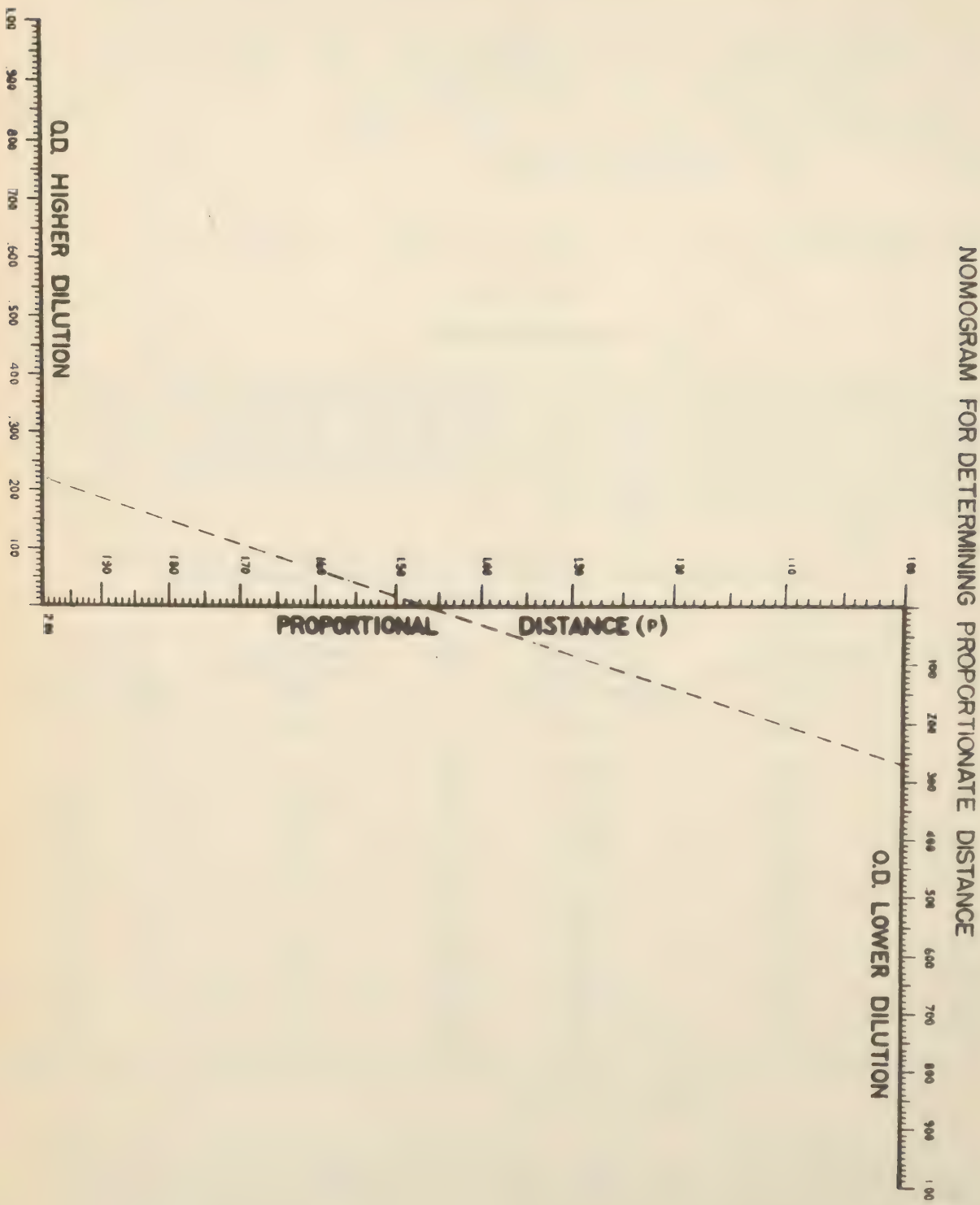
Table XIII. Antibody Units in Syphilitic Sera Estimated by the Quantitative Kolmer-Wassermann and the 50 Per Cent Complement Fixation Tests

Serum No.	Quantitative Kolmer-Wassermann Test	50 Per Cent Complement Fixation Test	Serum No.	Quantitative Kolmer-Wassermann Test	50 Per Cent Complement Fixation Test
	units	units		units	units
1	128	2446	14	16	242
2	128	1960	15	16	208
3	128	1880	16	8	192
4	64	1752	17	8	120
5	64	1376	18	3	80
6	64	952	19	3	76
7	32	657	20	3	76
8	32	650	21	1	58
9	32	595	22	1	52
10	32	344	23	0*	49
11	16	342	24	0*	37
12	16	262	25	0	27
13	16	235	26	0	12

* Trace of a reaction.

The capacity of the 50 per cent technic to detect fine differences in antibody levels was investigated next. Given luetic sera were initially diluted 1:16 with veronal-buffered saline. Then, using small but progressively widening increments, they were further diluted and each dilution was titrated in parallel by the quantitative Kolmer-Wassermann test and by the 50% method. Comparative results,

Figure 5


NOMOGRAM FOR DETERMINING PROPORTIONATE DISTANCE
O.D. HIGHER DILUTION
O.D. LOWER DILUTION
PROPORTIONAL DISTANCE (P)

typical of several experiments, are summarized in Table XIV. It can be noted that the 50 per cent test demonstrates superior sensitivity and more effective differentiation of antibody levels.

Specificity Studies

Information concerning specificity of the 50 per cent end point method was sought. Systems containing Kolmer-Wassermann antigen in the presence of various heterologous antisera were examined by the Kolmer-Wassermann and the 50 per cent procedures. Table XV. indicates the results obtained. It appears that the 50 per cent method is as specific as the Kolmer-Wassermann test. One serum from an epidemic typhus case gave an apparently non-specific reaction. It is unknown whether this individual, unavailable for further study, was also luetic.

Table XIV. Antibody Units Detected in Graded Dilutions of a Given Syphilitic Serum Estimated by the Quantitative Kolmer-Wassermann and the 50 Per Cent Complement Fixation Tests

Serum Dilutions	Quantitative Kolmer-Wassermann Test	50 Per Cent Complement Fixation Test	Serum Dilutions	Quantitative Kolmer-Wassermann Test	50 Per Cent Complement Fixation Test
1:	units	units	1:	units	units
16.0	8	128	31.4	2	59
17.1	8	120	34.5	1	55
18.4	8	118	43.2	0*	48
20.0	4	110	49.3	0*	40
20.2	4	101	57.5	0	33
21.5	4	97	69.0	0	29
21.8	3	96	86.2	0	26
22.9	3	95	115.0	0	22
24.6	3	88	172.0	0	12
26.4	2	68	345.0	0	<10
28.8	2	61			

* Trace of a reaction

Discussion

During the course of the present studies technical and theoretical difficulties were encountered. Some were minor in magnitude, others notably more significant. Each received independent examination. When apparently resolved, they were integrated into the main pattern of the investigation. Several of the technics and theoretical concepts warrant detailed consideration.

1. Early in the work the veronal-saline buffer described by Mayer and his coworkers (13) was employed. However, because the activity of complement did not remain satisfactorily stable in that diluent, a modification was sought. With incorporation of 0.30 per cent gelatin, it was observed that the activity was enhanced and relatively stabilized both at 4°C, the fixation temperature, and at 37°C, the incubation temperature. Moreover, inclusion of gelatin retarded spontaneous lysis of sensitized cells and resulted in a clear centrifuged supernate which aided accurate spectrophotometric determinations.

2. The concentration of sheep erythrocytes, the reagent volumes for the complement titration set and the source of complement duplicated those recommended by Kent (11). However, because of its enhanced activity when diluted in gelatin-veronal buffer, lyophilized complement was titrated at 1:200 rather than 1:100 dilution.

Table XV. Specificity of the 50 Per Cent Complement Fixation Test. Syphilitic Antigen-Heterologous Serum Systems

Description		Titer with Homologous Antigen	Quantitative Kolmer-Wassermann Test	50 Per Cent Complement Fixation Test	Remarks
			units	units	
Schistosomiasis	human		0	<10	
"	"		0	<10	
Jap B. Enceph.	horse	1: 16 CF*; 320 NT**	0	<10	
"	"	1: 64 CF; 1,000 NT	0	<10	
"	human	1: 32 CF; 32,000 NT	0	<10	
"	"	1: 16 CF; 63,000	0	<10	
"	"	1: 8 CF 5,000 NT	0	<10	
"	"	1: 32 CF; 100,000 NT	0	<10	
West. Equine Enceph.	guinea pig	1: 128 CF	0	<10	hyperimmune serum
St. Louis enceph.	"	1: 256 CF	0	< 10	"
"	"	1: 64 CF	0	< 10	"
Typhus, epidemic	human	1: 320 CF	0	< 10	possibly luetic
"	"	1: 160 CF	32	241	
"	"	1: 320 CF	0	< 10	
Typhus, endemic	"	1: 80 CF; 1:2560 Agg△	0	< 10	
"	"	1: 40 CF	0 ac[x]	< 10	
Tbc., intestinal, acute	"			< 10	
" pulmonary, acute	"			< 10	
" " "	"			< 10	
" " chronic	"			< 10	
" " "	"			< 10	
Brucella abortus	rabbit	1:5120	0	< 10	hyperimmune serum
Salmonella derby, anti H	"	1:5120	0	< 10	"
Salmonella minnesota, anti O		1:5120	0	< 10	"
Infectious mononucleosis	human	1:1792	0	< 10	
" "	"	1: 896	0	< 10	
Normal	"		0	< 10	
"	rabbit		0	< 10	
"	horse		0	< 10	
"	guinea pig		0	< 10	

* complement fixation; ** neutralization test; △ agglutination test; x anticomplementary

3. For all spectrophotometric determinations a wavelength of 550 mμ was substituted for the 580 mμ employed by Kent. This modification was adopted after a preliminary study indicated that less deviation from the Lambert-Beer Law (greater parallelism between optical density and expected per cent hemolysis) occurred at 550 mμ (21). Indeed, even at this optimal wavelength strict proportionality was not obtained, since the optical density for 50 per cent hemolysis exceeded half that obtained for 100 per cent hemolysis. It therefore seemed necessary, for corrective measures, to incorporate 50 per cent reference sets in the complement and amboceptor titrations.

4. At present the majority of workers applying the 50 per cent hemolytic unit to complement fixation tests exhibit a trend toward utilization of relatively large quantities of complement. Thus, Kent (22) recommended three complement units for the serodiagnosis of amebiasis. Wadsworth, Maltaner and Maltaner used 3, 6, 9 and 12 units in tests for syphilis, gonorrhea and tuberculosis. Varley and Weedon (23) also preferred multiple units for testing typhus fever serum as did Rice (24, 25) for titrating antipneumococcus and antivaccinia sera. Later, Maltaner and Gnesh (26) used 6K to determine titers of syphilitic sera up to 100 and Dulaney et al (27) selected 4K in testing for granuloma inguinale. More recently Mayer and co-workers (28, 29, 30, 31) carried out their quantitative studies with 50 to 200K. In 1949 Wolfe and Kornfeld (32) employed up to 7.5K in differentiating strains of Q fever.

In the opinion of the writers, the use herein of only 2K for the complement-fixation test invites critical consideration. An attempt will be made to justify this choice on theoretical grounds.

Regardless of the technics practiced, all complement-fixation studies employing the end point of 50 per cent hemolysis rests on a fundamental requirement. A measurable quantity of the serum under examination must in combination with homologous antigen bind the available complement incompletely, leaving the remaining unfixed complement free to effect partial hemolysis of the sensitized erythrocytes. Hemolysis falling within the limits of a 5 to 95 per cent range is essential for accuracy. The amount of serum needed to give 50 per cent hemolysis can then be determined by graphs, tables, monograms or other means.

The von Krogh formula, discussed earlier in this paper permits calculation of the conditions required for partial hemolysis.

If x is expressed in complement units, formula (II) becomes

(VII) $$\log x = \frac{1}{n} \log \frac{y}{1-y}$$

For $y = 5\%$ and $1 = 0.200$,

$$\log x = 0.200 \log \frac{5}{95} = 0.256$$

and $x = 0.56$

For $y = 95\%$

$$\log x = 0.200 \log \frac{95}{5} = 0.256$$

and $x = 1.80$

Hence, the acceptable limits of partial hemolysis will result when 0.56K to 1.80K are available.

Wadsworth and subsequently Rice (25) have demonstrated that in the presence of an optimal concentration of antigen the quantity of complement fixed is proportioned to the antibody content of the serum. It follows therefore, that if 12K are used in a complement fixation test, 5 per cent hemolysis will be produced when the antiserum in the reacting system contains just 11.44 antibody units. Similarly, 95 per cent hemolysis will be produced when the antiserum contains 10.20 antibody units.

In diluting antiserum serially for titration the dilution increment should be such that at least one dilution will give hemolysis falling between the 5 and 95 per cent points. Actually, it is preferable if two or more successive dilutions do so.

The advantage of using a lesser number of K in the complement fixation test are demonstrated in Table XVI.

The antibody units listed in columns (b) and (c) were obtained by deducting 0.56K and 1.80K respectively from the K values recorded in (a). The fourth and fifth columns indicate the maximal common dilution factor essential for realization of partial hemolysis, in one and in two tubes.

The data show that the common dilution factor varies inversely with the quantity of K used. With multiple units of complement, this factor is strikingly small, while with two units the simple two-fold dilution scheme becomes applicable.

In illustration, if 12K are employed to titrate a serum containing 120 antibody units in the first tube, to obtain two tubes in the dilution series giving partial hemolysis, at least 41 dilutions would be required. Logically this would impose need for a preliminary or "screen" titration. With 2K, however, two-fold dilutions can be used and partial hemolysis will fall in the 7th and 8th tubes.

Table XVI. Relation of K to the Common Dilution Ratio Required for Partial Hemolysis

K Used in Test	Antibody Units Necessary For 5% Hemolysis	Antibody Units Necessary For 95% Hemolysis	Maximal Common Dilution Factor Required for Partial Hemolysis in 1 Tube $(\frac{b}{c})$	Maximal Common Dilution Factor Required for Partial Hemolysis in 2 Tubes $(\sqrt{\frac{b}{c}})$
(a)	(b)	(c)		
12	11.44	10.20	1:1.12	1:1.06
9	8.44	7.20	1:1.17	1:1.08
6	5.44	4.20	1:1.30	1:1.14
4	3.44	2.20	1:1.56	1:1.25
3	2.44	1.20	1:2.03	1:1.43
2	1.44	0.20	1:7.20	1:2.68

Further examination of Table XVI reveals a fact of considerable significance. The use of 2K promotes substantially superior sensitivity. The 2K system detects some fixation of complement with 0.20 antibody units whereas the 9K system, for example, fails to demonstrate fixation with serum of less than 7.20 antibody units.

7. The method of titrating antigen described in this report does not deviate in principle from basic precepts. Determination of the optimal concentration of antigen, however, is more rigorous but perhaps more precise. Full exploitation of the greater accuracy provided by the spectrophotometric permits reliable construction of curves of hemolysis. The optimum is determined at the 50 per cent zone thus leading to enhanced precision and to the maintenance of a criterion of measurement which has been sought throughout this investigation.

An observation has been made which may be worthy of attention. Although the importance of ageing antigen is well known in certain flocculation tests for syphilis, it seems to have been overlooked in complement fixation tests. Experience in this laboratory has suggested that the activity of fresh-diluted antigen undergoes a rapid change but becomes more stable after three hours. Disregard of this fact may induce considerable variations in antibody assays of a given serum due to alterations in antigen-antibody ratio.

In comparison to the antigen concentrations employed by other workers, those used herein are considerably more diluted. This is an expected consequence of the fact that with only 2K, less antibody is required for fixation. Hence, less antigen is needed to preserve the optimal antigen-antibody ratio.

8. The greater sensitivity afforded by the use of 2K and the consequent reduction in homologous antigen and antiserum required to demonstrate reactivity possibly minimize nonspecific reactions. It is conceivable that non-specific factors may thus be diluted out, resulting in enhanced specificity.

In the complement fixation test the comparison of optical densities between corresponding (rear and front row) tubes possesses an advantage. Each tube containing test serum and antigen has an almost exact counterpart in all respects but antigen, which is highly diluted in the test series and absent in the control series. Thus, should the serum be anticomplementary, chylous, icteric, or hemolyzed, correction for these undesirable qualities is automatically made.

The complement fixation test described in this communication invites more exhaustive applications than have been reported here. Nevertheless, preliminary results with luetic sera suggest that it may be an aid to definitive serologic studies, especially those rendered equivocal by prevailing methods.

Essentially, the test is a measuring device of relatively high sensitivity and precision. These properties may promote detection and quantitation of circulating antibodies present early in disease as well as during acute phase and convalescent periods. Demonstrable differences in antibody content could then be readily correlated with changes in disease status.

The test recommends itself to careful studies in luetic therapy. The efficacy of antibiotics may be better established by precise determinations of antibody levels at various stages of treatment.

Application to epidemiologic investigations is suggested. Often, the low grade immunity in individuals exposed to subclinical and abortive infections can not be distinguished by conventional tests from that of unexposed persons. Conceivably, a more sensitive measure of antibody levels would differentiate convalescent low-titer sera from normal sera. By this means given populations might be classified as having undergone or been free from past epidemics and endemics.

The specificity merits of the test have not been fully exploited. However, available evidence suggests that the use of high dilutions of antigen and antiserum tends to lessen non-specific and cross reactions. In consequence, more effective separation of immunologically related diseases as well as finer distinction between strains of a given disease might be achieved.

Summary

A quantitative complement fixation test employing the unit of 50 per cent hemolysis has been described in detail.

In practice the test proved highly sensitive, specific and relatively simple to carry out.

Only two 50 per cent hemolytic units were used. This contrasts with the multiple 50 per cent units recommended by other investigators.

The underlying advantages and possible applications of the test have been discussed.

CHEMISTRY SECTION

The section functions as a Chemical Laboratory serving the Far East Command. Its major functions can be listed as follows:

A. Clinical Chemistry
B. Toxicologic analysis
C. Water Chemistry
D. Miscellaneous analysis
E. Research
F. Training

The routine work performed showed an overall increase of roughly 60 per cent as compared with the preceding year.

Table I. Routine Chemical Examinations

	Clinical Chemistry		Toxicology		Water Chemistry		Miscellaneous Analysis		Totals	
	Samples	Tests	Samples	Tests	Samples	Tests	Samples	Tests	Samples	Tests
1948	2751	3889	276	5520*	129	1828	500	1201	3,656	12,438
1949	3860	5593	357	1660	168	3978	1461	2743	5,846	13,974

* Method of computation unknown.

CLINICAL CHEMISTRY: The clinical chemistry section performs all the various analyses requested by Medical Corps officers assigned to hospitals and dispensaries within the Far East Command. These determinations primarily serve as a tool aiding in the diagnosis and progressive course of diseases.

The kind and number of determinations received by the section is dependent upon the available facilities of the various hospital and dispensary laboratories. This varies from all clinical chemistries from some nearby installations, to only highly technical examinations, or those requiring rare or expensive equipment or reagents.

The monthly total of the different kinds of specimens received is given below in Table II.

Table II. Clinical Specimens Received in 1949

Specimen	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Blood	259	206	291	248	245	204	225	264	275	282	301	250	3050
Feces	17	25	9	21	13	22	14	36	13	18	7	9	204
Spinal Fluid	25	43	46	30	33	49	24	51	67	89	51	32	540
Urine	5	7	5	5	1	9	6	7	7	3	1	2	58
Others						5				1	1	1	8
Totals	306	281	351	304	292	289	269	358	362	393	361	294	3860

The above table shows that 79 per cent of all clinical specimens were bloods. Mention should be made that 1307 blood specimens submitted for alcohol determinations have been included in this value. Spinal fluids and feces places second and third with only 14 and 5 per cent respectively.

Table III gives the number and types of determinations performed on the above-mentioned specimens.

Table III. Determinations Performed in Clinical Chemistry

Acetone (Qual)	3	Cholesterol		Lipids	3	Prothrombin time	8
A/G ratio	144	Total	235	Non-protein nitrogen	164	Salicylates	6
Albumin	146	Esters	33	Occult blood	160	Specific gravity	3
Alcohol	1307	Creatinine	15	pH	6	Sodium	49
Amylase	50	Fat (fecal)	42	Phosphatase		Sulfanilamide levels	6
Bile	17	Fatty acids (fecal)	3	Acid	19	Thiocyanate	5
Bilirubin	334	Globulin	141	Alkaline	57	Thymol turbidity	408
Bromosulphalein	49	Globulin (Qual)	143	Phenolsulfonephthalein	20	Total protein	703
Calcium	48	Glucose	290	Phosphorous	27	Urea nitrogen	114
Carbon dioxide	12	Glucose Tolerance	38	Porphyrin	2	Urea clearance	2
Cephalin flocculation	539	Hemoglobin	3	Coporphyrin	1	Uric acid	51
Chemical analysis of calculus	2	Icterus Index	196	Uroporphyrin	1	Urobilinogen	6
Chlorides	44	Lipase	10	Potassium	24	Vitamin C	9

Total 5593

A better idea of the facilities afforded is given by a study of examinations performed (Table III).

During the year there were several changes in Standard Operating Procedures. New procedures were introduced and old ones reviewed, evaluated and revised when changes were indicated. These were:

A. A standard operational procedure for lead assays was introduced. The method from Simmons and Gentzkow (1) based on a colorimetric dithizone reaction was introduced.

B. A procedure for lipids (2) utilizing oxidation of lipids with dichromate and then titrating the excess dichromate with thiosulfate was initiated.

C. Thiocyanate levels which were requested were determined according to a published method (3) based on the reaction of the thiocyanate radical with ferric nitrate.

D. Increased requests for sodium and potassium made it feasible to investigate less time-consuming procedures. A micro method for sodium (4) was set up using only 0.2 ml. of the serum. The principle of the reaction is based on the precipitation of an insoluble salt of sodium with potassium pyroantimonate with subsequent iodiometric titration of the penta-valent antimony. The method for potassium (5) is based on the precipitation of potassium as the cobaltinitrite salt which is then diazotized with sulfanilamide. This produces a characteristic color measurable spectrophotometrically.

E. The one request for phospholipids was done according to the method described in Hawk et al (6). This consists essentially of extraction of the lipids, decomposition of the extract by acid digestion and estimation of the liberated phosphate by the standard phosphomolybdate method.

F. Micro methods for sugar (7) and total proteins in spinal fluids were initiated. The method for total protein makes use of 0.3 ml instead of the 2.0 ml generally used. The protein is precipitated with sulfosalicylic acid and the turbidity is read spectrophotometrically.

G. A simple procedure for amylase (8) was instituted replacing an older method. The analysis is performed by serial dilutions of the serum and incubated with 0.1 per cent starch solution to determine at what dilution the serum can maintain diastic action.

H. Ducci's colloidal red test (9) was inaugurated as a supplement of the tests already performed in evaluating liver dysfunction. It is stated that values higher than

two plus indicated abnormality. The results obtained with the colloidal red test do not necessarily correlate or correspond with those of thymol turbidity and cephalin flocculation. Normal values can be considered to be less than 3 for the colloidal red test, 0 to 4 units for thymol turbidity, and 0 for the cephalin flocculation test.

I. Steps have been taken to inaugurate the determination of iodine (10) in blood recently reported by O'Connor et al.

TOXICOLOGY: It has been the duty of the chemistry section to perform toxicological analysis on selected autopsy tissues and fluids submitted. These facilities are available to persons serving as Medical Examiner for any branch of the National Military Establishment in FEC. In certain few instances, similar service has been afforded local British Commonwealth Occupation Forces. In most of the cases under consideration by the section death occurred under unusual circumstances requiring coroner type investigation. In most of the cases, specimens were accompanied by sufficient information to clarify the chemical approach of the examination.

There was no way of predicting the amount of work which would present itself, but specimens were submitted in a steady stream throughout the year. It was important to have only trained personnel assigned to this section as all its assays dealt with matters which might come before law courts. In all important questions, guidance has been sought from medical authorities.

A total of 131 autopsy cases submitted for toxicological analysis were completed during the calendar year. The largest number (100) of requests have been made by the Pathology group of this unit. This is an increase of 36 cases or 38 per cent over the work load performed in 1948. Cases per month varied from 8 to 19, and for the year one case submitted every third day. It should be mentioned that inasmuch as investigative efforts and possible criminal action are occasionally dependent on such reports, every effort must be made to effect expeditious completion of the examinations.

A complete list of body fluids and tissues submitted on the above 131 cases is given in Table IV.

The type and number of determinations performed on the above 357 specimens is given in Table V.

Table IV. Body Tissue and Fluids Submitted for Toxicological Analysis

Tissue or Fluid	No. Received
Blood	104
Brain	70
Bronchial contents	1
Kidney	2
Liver	79
Pericardial fluid	2
Pleural fluid	1
Spinal fluid	4
Spleen	1
Stomach contents	57
Urine	36
Total	357

Table V. Determinations on Body Fluids and Tissues Submitted for Toxicological Analysis

Determination	No. Tests
Alcohol, ethyl	202
Alcohol, methyl	198
Alkaloids	179
Barbiturates	185
Carbon dioxide	4
Carbon monoxide	6
Chlorides	42
Cyanides	164
Fluorides	3
Halogenated compounds	164
Heavy metals	165
Magnesium	2
Morphine	175
Paraldehyde	1
Phenols	164
Phosphorous	3
Sugar	1
Total	1660

A brief review of the significant positive findings may be of interest.

Ethyl Alcohol - In general this substance was most commonly discovered in this group of examinations being found in 23 per cent (30 cases out of 131) of all autopsy subjects in significantly excessive concentrations. Maximum levels obtained were:

Gastric Contents	19 mg/ml
Brain	3.9 mg/ml
Liver	2.6 mg/ml

Methyl Alcohol - This substance was found four times. In three instances stomach contents shows 0.03, 0.06, and 6.0 mg/ml. In a fourth instance the liver contained 0.05 mg/ml.

Chlorides - Comparison of chlorides in blood from right and left ventricles was made in 9 instances. Of these, significant findings (more than 100 mg/100 ml higher in left ventricle) supported the finding of death by drowning in salt water in three instances with one additional probable case.

Carbon Monoxide - This substance was found twice. (1) Case J-655 - 18.5 per cent of blood saturation. (2) Case J-933 - 89.0 per cent of blood saturation.

Barbiturates - Phenobarbital was found to be present in the liver of case J-712.

Paraldehyde - This substance was found in the gastric contents of case J-641.

Phenols - A trace of phenolic compounds was found in the brain of case J-655. However, the presence of phenolic compounds cannot be considered significant since none was found in the liver.

WATER CHEMISTRY: Of 168 samples on which analysis and report was completed, 88 were received at the close of 1948. With minor fluctuations, samples were submitted at the rate of 10 per month. This required 3978 examinations using essentially the procedure indicated previously (11).

Of interest are those potable waters which exceeded the maximum color and turbidity values as given in "Drinking Water Standards for Interstate Carriers" (12). These maximum values are 10 and 20 for turbidity and color, respectively. The location of these non-complying potable waters and the values obtained are given in Table VI.

Table VI. Potable Waters which Exceeded Maximum Turbidity and Color Requirements

Source	Turbidity	Color
Tachikawa Air Force Base	20	
8th Army Engineers, Wajerio Wells	25	30
8th Army Engineers 11th Mess Hall	15	
Deep Well at Sakata	200	90
Itazuke Air Force Base, Well #1		42
Itazuke Air Force Base, Well #2		60
Itazuke Air Force Base, Mikasa River	30	
Regional Post Engineer, Well #6	75	75
Camp Matsushima	40	
U. S. House #717	90	
Otsu City	50	
Kizu Intake	80	
Camp Sakai, Deep Well Pump	95	27
Marianas-Bonins Command (Main Well)	30	
584th Engineers, River	50	30
8th Cavalry, Pump Station		44

Eighteen percent (16 out of 87) of the potable waters analyzed did not comply with either turbidity and/or color as specified in "Drinking Water Standards for

Interstate Carriers" mentioned above. However, of equal importance is the fact that all potable waters submitted contained considerably less than the maximum allowable quantities of sulfate, magnesium, total residue, and chlorides. In Table VII is given the maximum allowable amount and an average of the 87 potable waters analyzed.

Table VII. Concentration of Normal Constituents in Japanese Potable Waters

Substance	Average (87 samples)	Maximum Allowable (U. S. Standards)
Sulfate	8	250
Magnesium	6	100
Total residue	123	1000
Chlorides	11	250

MISCELLANEOUS ANALYSIS: This term serves as a rallying point for a considerable variety of investigations performed by the section. The laboratory performs qualitative and quantitative analyses of any materials submitted to it by various military agencies, e.g., Medical Officers, Sanitary Inspectors, Technical Intelligence, Military Police, Counter Intelligence Corps, Engineers, Economic and Scientific Section, Civil Affair Teams, Public Health and Welfare and Quartermaster or Technical Supply Agencies. The laboratory identifies unknown materials. In addition, this section analyzes food products and beverages which are suspected of having caused sickness or disease, or for other reasons felt to be probably contaminated. Routine check of food stuffs for compliance with purchase specifications is not performed. Any questionable item submitted by a member of the Veterinary Corps with request for specific examination is accepted.

At times these analyses can be fitted into routine work, but in most instances, each analysis poses a separate problem. They illustrate the unpredictable work which must be carried out by the Medical General Laboratory and the nature of the service which the chemistry laboratory must be able to offer other laboratories and investigative units.

Some idea of the variety and volume of requests submitted can be gained from the list of all the miscellaneous samples which were received during the calendar year grouped into rough categories given in Table VIII.

Table VIII. Miscellaneous Samples Analyzed During 1949

Category	Samples Analyzed
Alcohols and Beverages	330
Bacteriologic Products	19
Body fluids	48
Insecticides	17
Food Products	23
Household items	22
Medicinal Products	25
Milk and milk products	60
Oxygen	168
Unknown Materials	76
Water	10
Water Purification Materials	630
All others	34
Total	1462

Because each new request frequently constitutes an entirely new problem and method of approach the different analyses performed do not lend themselves readily to rigid classification. However, these analyses can be classified into the following main groups, namely, physical constants, toxicity tests, identification

tests, quantitative and qualitative analyses. It is believed that it is appropriate to list all different types of determinations performed under the above-mentioned general groups. This will give the reader a truer evaluation of what was actually accomplished. The types of analyses and number of determinations are given in Table IX. The actual determinations performed may again be roughly grouped for statistical summation (Table IX.).

Table IX. Determinations Performed on Miscellaneous Samples

General Type of Examination	Variety of Specific Tests	No. Tests Performed
Physical Constants	4	64
Biologic Toxicity	3	60
Identification Tests	54	907
Quantitative Tests	37	1373
Qualitative Tests	14	339
Total		2743

A brief discussion of the more interesting miscellaneous samples analyzed follows: Alcohols and Beverages - Twenty per cent, i.e., ten out of 50, of the whiskey samples submitted were condemned because one contained a moderate amount of fusel oil and the remaining nine contained methyl alcohol, ranging from 0.13 to 6.0 mg/ml. Those which were condemned were all of Japanese origin. In reviewing the data one interesting observation was made, that being, that the condemned samples (10 out of 19) were submitted during the first part of the year. Subsequent to June, however, the other 31 samples were found to meet the requirements set forth in Eighth Army Operational Directive No. 50, dated 17 May 1946.

Ten cases of American beer, two each from five lots, were submitted to the laboratory. A chemical analysis was requested to determine if the beer was "flat". Determination of carbon dioxide (13) showed that only one lot of the five contained a very low carbon dioxide content, namely, .0.13 per cent. This lot also tasted flat and did not give the characteristic foam when poured into a glass container. Allen (14) states that "A beer containing less than 500 cc (0.1 per cent) of CO_2 per liter is flat to the palate". The average carbon dioxide content of the remaining four lots ranged from 0.25 to 0.41 per cent.

Bacteriological Materials - The Bacteriology Section, 406th Medical General Laboratory, submitted several antigens, cultures, and media for nitrogen and pH determinations.

Food Products - In Table X below is given the results of the miscellaneous food products submitted during the calendar year. Two of the above samples submitted were of enough interest to be commented upon.

During the spring several Filipino laborers on a picnic on Saipan caught and ate a huge "eel". Shortly thereafter, they developed symptoms of severe neurotoxic poisoning, with two deaths. Blood and urine submitted at that time was negative. This laboratory requested submission of some of the suspect eel. In August an iced-specimen was received and subsequently identified as a type of "Gymnothorax" or Moray. When its flesh, cooked or raw, was fed to 6 monkeys, 10 rats, and 20 mice, it was found to be innocuous. Isolation from combined skin, fat, meat and bone resulted in negative findings. Japanese authorities recognize the fact that toxicity of the eels often varies from individual to individual within the species and is also probably seasonal.

Reddish-pink and reddish mottled beans of the kidney bean size and shape were submitted to the laboratory for analysis because they had caused 26 cases of diarrhea and 4 deaths to consumers. These beans had been distributed at several Japanese ration points. Such beans were found to contain cyanogenic compounds capable of liberating toxic amounts of HCN on digestion. Investigation reveals native Malayans, accustomed to such a diet, suffer no ill effects.

Table X. Miscellaneous Food Products Analyzed During 1949

Food Product	Requested or Suspected	Result
Beans, white, navy	Cyanide	Negative
Beans, reddish and mottled	Cyanide	Positive
Soy bean oil (2 samples)	Specific gravity	0.9378 0.9222
	Refractive index	1.5145 1.4749
	Iodine number	136.0 128.0
	Saponification number	229.0 222.0
	Acid number	16.2 2.5
Flour (6 samples)	Contaminated with quebracho	Negative
Bread	Sodium	32 mg/100 grams
	Moisture	37 per cent
Macaroni	Barbiturates and/or alkaloids	Negative
Doughnut mix	Carbon dioxide content	Negative
Doughnut mix	Soap or other adulterants	Negative
Milk	Barbiturates	Positive
Cheese	Butter fat	22 per cent (wet basis)
	Moisture	35 per cent
Baking powder	Carbon dioxide content	7.4 per cent
Lemon flavoring	Lemon oil concentration	94 per cent
Eel	Poisons	Negative
Scallops	pH	5.87
Oysters	pH	6.00
Juice	Alkaloids and/or barbiturates	Negative

Milk and Milk Products - A total of 60 milk samples were submitted to the laboratory for chemical analysis. The official methods as given in the 6th edition of the Association of Official Agricultural Chemists were used. On several occasions our butter fat results did not agree with those obtained by the commercial firm. It was learned that this disagreement resulted because the Chemistry Section was using the Roese-Gottlieb (17) method whereas the commercial firm used the less accurate Babcock method.

The specification for white recombined milk states that the product shall contain no less than 3.25 per cent butter fat and no less than 8.75 per cent solids not fat. Table XI gives the results of those samples analyzed in the laboratory. The table is given to show that it is possible to produce a suitable recombined white milk by mixing butter oil, skimmed milk and water in the right proportion.

The mean butter fat and solids not fat content of the samples in Table XI is 3.28 and 8.83 per cent, respectively. Although not shown in Table XI it should be mentioned that the specific gravity ranged from 1.029 to 1.032, the titratable acidity (calculated as lactic acid) was either 0.13 or 0.14 per cent, and all gave a sediment reading of Disc No. 1 which signifies freedom from dirt and carmalized lactose.

Fourteen of the fifteen chocolate milk drink samples complied with the two per cent specification requirement. Again it is desirous to present the results in tabular form to point out that milk so made from butter oil, chocolate, skimmed milk and water can be satisfactorily produced, on a large scale (Table XII).

The mean fat and total solids content of the samples given in Table XII is 2.30 and 16.70 per cent, respectively. Again, though not quite as obvious as white milk, it can be seen that the company was able to maintain good control of its product. The specific gravity of the above chocolate milk drink samples ranged from 1.045 to 1.055.

Table XI. Butter Fat and Solids Not Fat in Recombined Milk

% Fat	% Solids Not Fat	% Fat	% Solids Not Fat	% Fat	% Solids Not Fat
3.01	7.84	3.33	8.72	3.38	8.74
3.28	8.94	3.25	8.83	3.29	8.82
3.20	8.79	3.38	---	3.10	8.99
3.33	8.86	3.44	8.95	3.25	8.95
2.68	8.93	3.60	8.40	3.35	8.87
3.24	8.93	3.34	8.74	3.12	8.93
3.29	8.77	3.22	8.99	3.25	---
3.13	---	3.59	8.75	3.75	8.77
2.94	8.80	3.36	8.75	3.49	8.86
3.28	8.77	3.26	9.04	3.28	8.83
3.34	8.91	3.32	8.78	3.00	8.63
3.32	8.94	3.37	9.14	3.32	8.94
3.37	9.17	3.29	8.86		

Table XII. Fat and Total Solids Content of Recombined Chocolate Milk Drink

% Fat	% Total Solids	% Fat	% Total Solids	% Fat	% Total Solids
2.02	---	2.27	16.58	2.23	---
2.31	16.86	2.20	16.66	2.26	16.67
2.21	16.63	2.61	17.39	1.96	16.47
2.46	16.12	2.46	16.77	2.75	17.22
2.16	16.49	2.24	16.55	2.29	16.64

Oxygen - A check for purity of oxygen produced locally for Medical Department use has become routine. Thirty different lots, representing a total of 168 oxygen cylinders, manufactured by the Nippon Rika Kogyo Co., Tokyo, were submitted by the 5th Army Medical Depot, for chemical analysis. Only two of the 30 lots were found to contain less than the 99 per cent by volume of oxygen as specified in the Pharmacopoeia of the United States, 13th revision, p. 373. These were lots 2-1/49 and 3-7/49 which contained an average of 97.69 and 98.72 per cent by volume of oxygen, respectively. At least one cylinder from each lot was analyzed for contaminants and all of the 34 cylinders so analyzed were found to contain less than the maximum allowable amount of acids or alkalies, carbon dioxide, carbon monoxide, halogens, and other oxidizing substances.

Unknowns - Various liquids, powders and tablets were submitted to the laboratory for identification and for the detection of narcotics or drugs. Some were representative samples of presently stocked unlabeled supplies stored in various Armed Forces installations, others were taken by the Military Police from apprehended individuals, and still others were obtained from personnel who had taken them orally either accidentally or with the intent to commit suicide. All 25 liquids were positively identified, some being methyl alcohol, epinephrine, potassium soap in a mixture of ammonia and gasoline, kerosene, crude petroleum, chloroform, sodium p-aminobenzene-sulfonacetamide, salicylic acid dissolved in a mixture of alcohol and acetone, isopropyl alcohol, brake fluid, 1-phenyl-2 methylamino propane hydrochloride, and a mixture of phenol and ammonia.

Thirty of the 32 powders and solids submitted were positively identified whereas the remaining two were identified only as belonging to a class of compounds. This was the most that could be done with the meager amount of sample submitted for analysis. The ingredient(s) of the more important unknowns follows: heroin, saccharin, glucose, sulfathiazole, calcium hypochlorite, salicylic acid, acetylsalicylic acid, an enzyme having diastase activity, protein, laundry soap, tea, iron and aluminum silicate, and a mixture of sodium bicarbonate and starch. Marihuana was not found in either one of two samples submitted for analysis.

All tablets submitted with one exception were positively identified. The active ingredient(s) of those identified were sodium bicarbonate, aluminum hydroxide, sulfanilamide, sulfadiazine, a mixture of acteylsalicylic acid and acetophenetiden, boric acid, sulfapyridine, sulfaguanidine, N-methylcyclohexenylmethyl barbituric acid (Evipal), 5-cyclohexenyl-5-ethyl barbituric acid (Phandorn), and a vitamin pill which contained thiamine, riboflavin, iron and calcium. The remaining sample was only partially identified as a barbiturate but the melting point did not agree with any of those recorded in the literature available.

Water Purification Tablets - Analysis of a few lots of Halazone tablets showed that either partial or complete deterioration had occurred during storage. As a result of this preliminary work a survey of all lots presently stocked in FEC Quartermaster Depots was begun. Only 15 samples of all tested to date (617) meet the the requirement of 1 mg of active chlorine per tablet. Because of the extent of deterioration of these stocks, proper authorities have declared all lots showing 0.5 mg per tablets as acceptable until the shortage occasioned by extensive condemnation can be relieved. Such inferior tablets were to be issued with instructions for use of double amounts of the tablets.

Miscellaneous Samples - Other miscellaneous samples which could not be classified under any of the above categories are listed in Table XIII below.

Table XIII. Miscellaneous Samples

Sample or Specimen	Request	Findings
Crayons (2 samples)	Poisons	Negative for potassium permanganate
Chlorine comparator (Japanese)	Comparison with standards	Standards stronger than labeled
Cigarettes or cigarette butts (5 samples)	Marihuana, aspirin and/or heroin	Negative
Gas	Identification	Nitrogen
Fingernail	Arsenic	Negative
Fuel hose	Identification of scrappings	Black carbon, graphite, and thread-like fibers
Hair, human (2 specimens)	Presence of dye	Dye of the toluene-diamine type
Icterus working standard	Confirmation	-----
Jet fuel	Addition of dye to give final specific gravity of 0.833± 0.003	Added Sudan IV, final reading 0.833
Naphtha (4 samples)	Aromatic or aliphatic?	Aromatic of the benzol type
Radium cement	Radioactivity	Radioactive
Thermometer	Standardization	-----
Varnish	Identification	Low boiling point hydrocarbon
Veronal buffers (10 samples)	pH	----

EFFECT OF VITAMIN DEFICIENCY ON SUSCEPTIBILITY TO JAPANESE B ENCEPHALITIS VIRUS: Production of Deficiency by Exclusion of Vitamin from Synthetic Diet - Investigative work was started during the year to determine the susceptibility of vitamin deficient mice to Japanese B Encephalitis mouse brain virus. It was planned to use a synthetic diet which corresponded to the "Present Day Nourishment of the Japanese People" reported in 1948 by the Welfare Department, Public Health Section (18). The synthetic diet given in Table XIV was patterned after this Japanese survey.

Table XIV. Synthetic Diet for Vitamin Deficiency Studies

Basic Foods		Vitamin	
Casein (vitamin-free)	150 gm	Riboflavin .....10 mg	
Sucrose	755 gm	Pyridoxine .....10 mg	
Agar	20 gm	Ca pantothenate .....100 mg	
Salt mixture (19)	25 gm	Vitamin A & D, conc...10 drops	
Fat (Spry or Lard)	50 gm	Thiamine ..... variable	

Although a vast amount of work has been done, the problem of producing avitaminosis and yet preventing a high incidence of mortality in the vitamin deficient control groups remains unsolved. In general, the following procedure was followed in all three vitamin deficiencies studied thus far, namely thiamine, niacin, and calcium pantothenate. One group of mice, weighing over 10 grams, was given the synthetic diet partially or completely devoid of the vitamin under investigation. A similar group was given a diet which contained enough of the vitamin to prevent avitaminosis. Following an experimental feeding period of 14 to 17 days the mice on the vitamin diet were injected with varying concentrations of Japanese B Encephalitis mouse brain virus. The vitamin deficient mice were divided into two subgroups, one injected with the corresponding dosages of virus and the other was inoculated with the same volume of sterile physiological saline in order to produce similar intracerebral injury.

In order to reduce the high mortality in the vitamin deficient mice injected with physiological saline, various minor changes were made in the diet by varying the vitamin concentrations and avoiding, as much as possible, any changes in the basic foods. In Table XV is given a resume of these diet changes and resulting per cent mortality in the vitamin deficient mice inoculated intracerebrally with physiological saline.

It can be seen from Table XV that the absence of calcium pantothenate from the diet resulted in 76 per cent mortality, and a diet devoid of niacin produced approximately 50 per cent mortality. Corn meal was used because our supply of casein became exhausted. Whole milk was added to the diet to supply trytophane which the mouse is capable of converting into niacin but this did not decrease the mortality rate.

Encouraging results have been obtained with thiamine deficiency studies. For the purpose of our experiment it appears that the level of thiamine required by the mouse lies somewhere between 1 and 3 mg. A concentration of 1 mg produced 83 per cent mortality whereas those given 3 or 5 mg of thiamine per Kg of food survived. However, there is no assurance that 3 mg actually produces a thiamine deficiency. Preliminary experiments showed that anorexia was the first clinical sign of deficiency determined by the amount of food consumed per mouse per day. It was found that healthy mice ate an average of 2 to 3 grams per day whereas those mice that lost their appetite ate less than one gram.

PRODUCTION OF VITAMIN DEFICIENCY BY INOCULATION OF VITAMIN ANALOGS: In addition to the above work on avitaminosis another approach, i.e., the administration of antivitamins, was attempted in order to reduce the high mortality rate of niacin deficient mice injected with physiological saline. The limited quantity of 3-acetylpyridine synthetized in the laboratory, was sufficient for a preliminary experiment. The above

Table XV. Vitamin Deficient Mice Inoculated Intracerebrally with Saline

Minor Changes in Diet*	No. Mice Inoculated	No. Mice Survived	% Mortality
(A) Calcium Pantothenate Deficiency			
Calcium pantothenate - none Thiamine - 10 mg	47	11	76
(B) Thiamine Deficiency			
Thiamine - none	318	55	82
Thiamine - none alpha tocopherol - 3 mg	199	4	98
Thiamine - 1 mg alpha tocopherol - 3 mg	18	3	83
Thiamine - 3 mg alpha tocopherol - 3mg	20	20	0
Thiamine - 5 mg alpha tocopherol - 3 mg	19	19	0
(C) Niacin Deficiency			
Caesin - none Corn meal - 150 gm Thiamine - 10 mg	95	43	55
Caesin - none Corn meal - 80 gm Whole milk, dry - 70 gm Thiamine - 10 mg	47	19	60

* All quantities are given as per Kg of synthetic food

basal diet was modified slightly as follows: 140 grams of whole milk powder and 10 grams of corn meal was substituted for the caesin, 200 mg of niacinamide was added to the diet. Thirty-five mice were given this diet for approximately 18 days. Of this number two groups of 15 mice each were injected with 0.05 ml (1.0 mg) and 0.10 ml (2.0 mg) of 3-acetylpyridine hydrochloride, the remaining five were used as controls. The first dose administered subcutaneously was followed by daily intraperitoneal injections. The results are given in Table XVI.

Table XVI. Weight Studies and Survival Rate of Mice Injected with 3-acetylpyridine hydrochloride

Group	Prior to Experiment	7th Day	12th Day	18th Day	% Loss
(A) Average weight (grams)					
Control	11.1	10.6	10.7	10.2	8
3-acetylpyridine					
0.05 ml (1.0 mg)	10.9	10.2	10.0	9.6	12
0.10 ml (2.0 mg)	10.7	11.0	10.6	10.6	1
(B) Survivors					
Control	5	5	4	3	40
3-acetylpyridine					
0.05 ml (1.0 mg)	15	13	13	9	40
0.10 ml (2.0 mg)	15	10	8	4	73

Prior to discussing the data in Table XVI it is worthwhile to mention that two days after the initial subcutaneous administration of 3-acetylpyridine hydrochloride 10 of the 15 given 0.05 ml (1.0 mg) and 8 of the 13 mice given 0.10 ml (2.0 mg) developed a lesion at the site of the injection. Hence it was necessary to change from a subcutaneous to an intraperitoneal administration. Surprisingly, no lesions occurred at the second site of inoculation. Evidence of healing of the lesions caused by subcutaneous injections was noted on about the 12th day.

It is apparent from the above table that 0.10 ml (2.0 mg) of 3-acetylpyridine administered parenterally daily to mice is too severe as 73 per cent (11 out of 15) died at the end of the 18th day. The one per cent weight loss which occurred is misleading because as the smaller mice died the average weight of the survivors increased. The control and those mice injected with 0.05 ml (1.0 mg) showed the same mortality rate, i.e. 40 per cent, and approximately the same weight loss, 8 and 12 per cent, respectively, at the end of the 18th day. It is believed that the mortality rate of the control mice would be decreased considerably if more mice had been used as controls. Also, it appears that a smaller dose, for example, 0.5 mg of 3-acetylpyridine, would cut down the mortality rate of the injected mice.

In another experiment, oxythiamine, an anti-vitamin of thiamine was also synthetized in the laboratory. Nine monkeys were fed a rice diet for 80 days at which time they were injected with 0.04 grams of oxythiamine over a period of seven days. The immediate effect of this material was to produce immediate inaction and dullness within one day after initial administration. Four of the group were given 10 mg of thiamine at the same time. The group receiving only oxythiamine died in less than nine days. Two of the group receiving thiamine in addition to the oxythiamine were still alive at the end of this period. Partial paralysis and convulsions were noted in two of the monkeys injected with oxythiamine. It can be concluded that oxythiamine is toxic to deficient monkeys at the level injected.

It can be concluded that:

1. Complete absence of either thiamine, niacin, or calcium pantothenate from the synthetic diet produces an avitaminosis of such severity that well over 50 per cent mortality results in those mice fed for three to four weeks on such a vitamin deficient diet.

2. Three mg of thiamine per Kg of synthetic food seems to be an adequate amount to prevent avitaminosis whereas 1 mg is not adequate to sustain life for any prolonged period of time.

3. Daily injection of 1 and 2 mg of 3-acetylpyridine to mice on a synthetic diet containing 200 mg of niacinamide per Kg of food results in 40 and 73 per cent mortality, respectively, at the end of 18 days.

4. Oxythiamine is toxic to thiamine deficient monkeys injected with 0.04 grams of the anti-vitamin over a period of seven days.

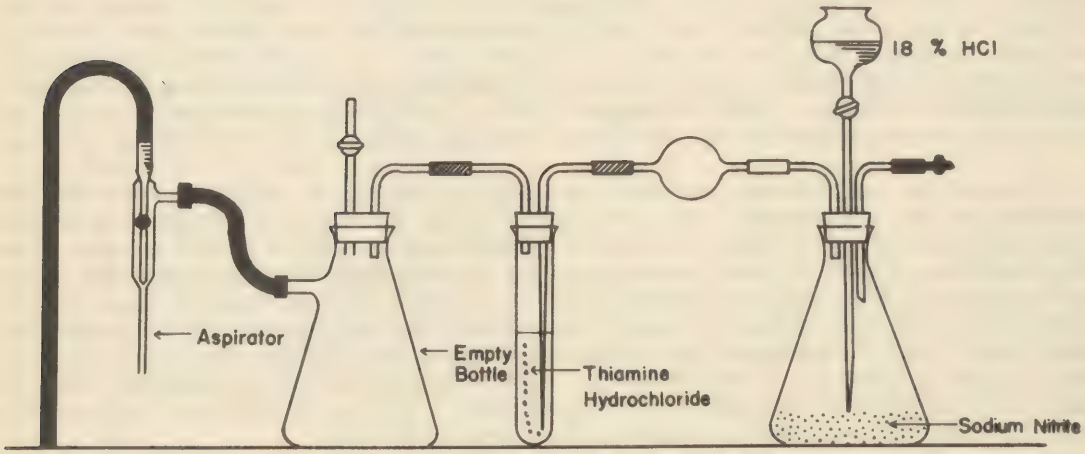
Synthesis of Vitamin Analogs - Oxythiamine - A synthesis of oxythiamine, a compound in which a hydroxyl group replaces the amino group of thiamine, has been described by Sodak and Cerecedo (20), who reported that the compound showed a toxic effect to mice which received a thiamine low diet, and that it inhibited the action of the enzyme of carp which destroys thiamine.

The procedure of preparing oxythiamine according to the method of Sodak and Cerecedo was as follows: The nitrous acid gas was generated in a flask which was open to the atmosphere through a small aperture, by the gradual addition of 18 per cent hydrochloric acid to solid sodium nitrite. It was passed under slight suction through an aqueous solution of thiamine hydrochloride. The nitrous acid gas was passed until the thiamine solution was saturated with it and showed a light green color. Then the reaction solution was evaporated in vacuo on the water bath at 60°C until an oil was obtained. After addition of a small amount of absolute alcohol, the solution was evaporated once more. The oil was dissolved in absolute ethyl alcohol and to the solution ether was added until precipitation was complete. The precipitate

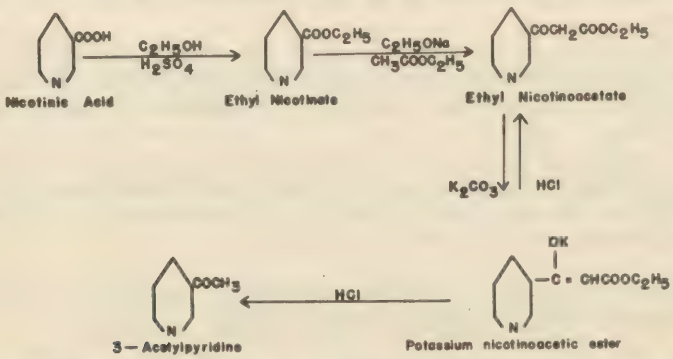
was then taken up in absolute alcohol and dry hydrogen chloride gas passed through the solution until it was saturated. A small amount of a white crystalline product was obtained. Upon addition of absolute ether to the solution more crystals separated out. The precipitate was washed with absolute alcohol and dried. The product melted at 195-197°C with decomposition, in agreement with the product obtained by Sodak. Figure 1 illustrates the apparatus used in the preparation of this analog of thiamine.

Figure 1

Apparatus Used for Synthesizing Oxythiamine



3-Acetylpyridine - Discussion - 3-Acetylpyridine is the nicotinic acid analogue which induces niacin deficiency in mice. This compound, because of its chemical similarity to niacin, probably acts by competition with the vitamin in physiological enzyme systems. The most available route of synthesis of this compound is as follows.



The first step, the conversion of nicotinic acid to its ester, is an easy one. This preparation can be achieved in a similar manner to the procedure used in conversion of an organic acid into its ester. Ethyl nicotinate is produced in good yield.

The second step, the condensation of ethyl nicotinate with ethyl acetate by the action of solid sodium ethoxide into nicotino-acetic ester, is the most difficult reaction in the synthesis of acetylpyridine. First of all, in this reaction it is necessary to use completely dry materials and vessels, and maintain an anhydrous atmosphere. In the first preparation, the procedure of Woolley(21) was used, but it was found impossible to dry the ethereal extract of nicotino-acetic ester with anhydrous potassium carbonate, because a large amount of fine needles was unexpectedly obtained when anhydrous potassium carbonate was added to the ethereal

solution of the nicotino-acetic ester. Therefore, the ester was converted to its potassium derivative, and then recrystallized from acetone as used by Pinner (22).

The third step, the hydrolysis of nicotino-acetic ester to 3-acetylpyridine is also a simple reaction.

Preparation of Ethyl Nicotinate - This preparation was carried out according to the description given by Camp (23). Twenty gm of nicotinic acid, 40 gm absolute alcohol and 40 gm concentrated sulfuric acid were heated on a water bath under a reflux condenser for 3 hours. The product was diluted with 3 volumes of ice, decomposed with sodium carbonate solution, and extracted with ether. After drying the ethereal extract with solid potassium carbonate and evaporation of ether an oily liquid was obtained. The oily liquid was then distilled under ordinary pressure. Nearly all the oil passed over at 223-224°C.

Preparation of Potassium Salt of Ethyl Nicotinoacetate - A mixture of 24 gm of ethyl nicotinate, 27 gm of ethyl acetate, and 16 gm of sodium ethoxide was allowed to stand at room temperature for about one hour with occasional shaking. The mixture became quite warm and assumed a deep reddish brown color. It was then refluxed five to six hours, cooled, and diluted with an equal volume of water. The unreacted esters were extracted with ether and the remaining aqueous solution acidified with concentrated hydrochloric acid and then made slightly alkaline with sodium carbonate solution. The oily layer of ethyl nicotino-acetate and acetoacetic ester was separated and the aqueous layer extracted with ether twice. These ether extracts were combined with the keto-ester layer and an oily liquid was obtained after ether was expelled. When concentrated potassium carbonate solution was added to the oil a large amount of fine needles was produced. The crystals were recrystallized from acetone-alcohol mixture.

Preparation of 3-Acetylpyridine Hydrochloride - A mixture of 15 gm of ethyl potassium nicotinoacetate, 30 ml of concentrated hydrochloric acid and 90 ml of water were refluxed for 6 hours. The resultant solution, which gave no coloration with ferric chloride, was evaporated to dryness on a steam bath and the residue recrystallized from alcohol. The product melted at 176°C as given by Woolley (21).

BARBITURATE ANALYSIS: The investigational work started by M. Balikov (24) in 1948 for devising a quantitative method for determining barbiturates in body fluids and tissues is still being studied. However, a procedure for the determination of barbiturates in simple solutions has been devised. The method is given below.

Principle - Barbiturates react with cobaltous ion in an anhydrous alkaline medium to give a pink color which can be measured spectrophotometrically.

Reagents - Cobaltous acetate solution, 1.1 per cent: Dissolve 1.1 grams of $CoAc_2.4H_2O$ in absolute methyl alcohol. This reagent is stable for 8 hours.

Isopropylamine, 33 per cent: To a 100 ml volumetric flask containing 33 ml of isopropylamine add absolute methyl alcohol to the mark. Store in refrigerator. This reagent is stable for 8 hours.

Barbital Standard: Dissolve 0.0500 gm of dried barbital in chloroform and make up to 100 ml in a volumetric flask. Each ml contains 0.5 mg of barbital.

Preparation of C-T Curve -

1. Dry curvettes by rinsing once with alcohol, twice with chloroform, and then allow to drain.

2. Place 2, 4, 6, 8 and 10 ml of standard barbital solution into five different curvettes. Dilute to 10 ml with chloroform where necessary. To a sixth curvette add 10 ml of chloroform.

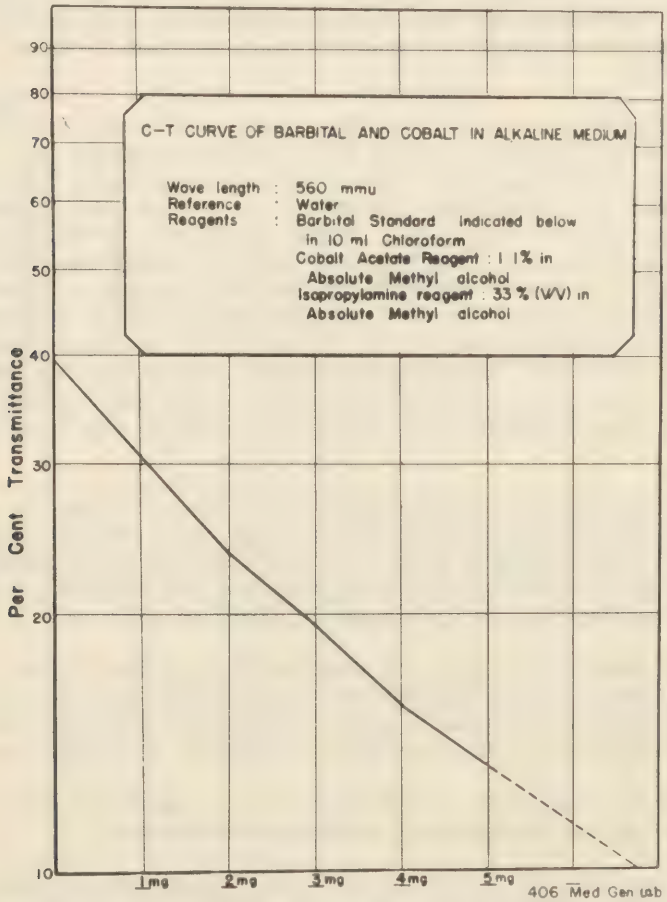
3. Pipet 1 ml of $CoAc_2$ reagent so as to form two layers.

4. Pipet 1 ml of isopropylamine so as to form a third layer. Wait exactly 5 minutes and then mix by inverting. Immediately read at 560 mu against a water blank.

5. Draw the C-T curve.

A typical C-T curve is given in Figure 2.

Figure 2



Koppanyi's (25) result was verified when a curve, instead of a straight line, was obtained when per cent transmittance was plotted against different concentrations of barbital. Thus at least five points are necessary to draw the reference curve.

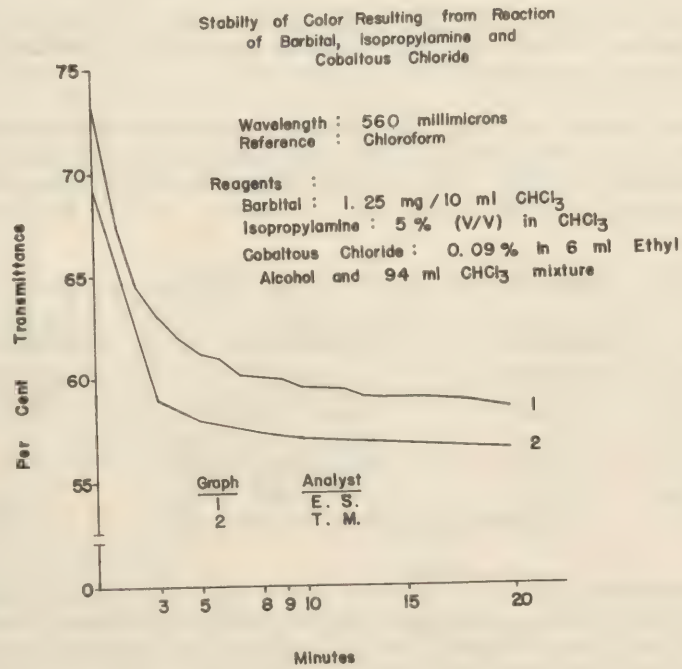
The limits of the sensitivity of this test is dependent upon the variability of the reagent blank. This has been found to vary within 0.2 mg of barbital. However, to include a substantial safety factor, the sensitivity can be stated as follows: Values of 0.2 - 0.5 mg of barbital are doubtful in that the reagent blank may be responsible for the reading; values less than 0.2 mg cannot be distinguished from a negative sample. Preliminary work indicates that a variation of precision occurs with concentration. Approximately 10 per cent error occurs when measuring quantities of 1 mg of barbital whereas only 2 per cent error occurs when the concentration of barbital is 5 mg. It is a fact that both sensitivity and precision will largely depend upon the efficacy of the extraction from tissue.

The above method was applied to body tissues containing known amounts of sodium barbital. To date, two methods have been tried: first, a simple acetone extraction was not suitable as less than 50 per cent recovery was obtained; and,

secondly, the continuous ether extraction based on the method of Kozelka et al (26) gave approximately 75 per cent recovery.

It was observed that a turbid solution resulted in the above method for determining barbiturates in simple solution. This may explain, at least in part, the low recoveries obtained from tissue. To eliminate this turbidity two changes were made in the method, they being: chloroform instead of methyl alcohol was used as the solvent for the cobaltous ion and cobaltous chloride was substituted for cobaltous acetate. A slight amount of ethyl alcohol (0.2 ml per mg $CoCl2$) was added to the chloroform to raise the solubility of the cobaltous chloride. However, an excess of ethyl alcohol caused a slight turbidity in the final color. The final color when chloroform was used as a solvent was very stable, color development reached a peak in 10 minutes and the solution remained clear for about 20 minutes. Figure 3 illustrates the stability of the final color.

Figure 3



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The modified procedure presented another problem, i.e., it was difficult to duplicate the preparation of a solution containing the same concentration of cobalt per unit volume. This was due to the hydroscopic character of cobaltous chloride. Thus the reagent was made by dissolving the anhydrous salt in absolute alcohol and diluting this so-called stock solution with a sufficient amount of chloroform. In order to keep the variables at a minimum a method for determining the concentration of cobalt in this reagent had to be simple, quick, and reliable. A spectrophotometric method is being investigated. However, prior to any studies on a C-T curve of cobaltous chloride dissolved in a mixture of alcohol and chloroform it was necessary to determine if an aqueous solution of cobaltous chloride obeyed the Beer-Lambert Law. Investigations revealed that the C-T curve of an aqueous solution of $CoCl_2$ does obey the Beer-Lambert Law with maximum absorption of light occurring at

510 mu, concentrations up to 1.2 grams of cobalt chloride dissolved in any aqueous medium can be determined spectrophotometrically, and a standard stock solution can be prepared directly from the hydrated salt ($CoCl_2.6H_2O$) without the use of gravimetric methods. The latter was determined by analyzing a 2 per cent $CoCl_2$ solution, prepared by dissolving 3.64 gram of $CoCl_2.6H_2O$ in water and diluted to 100 ml. The concentration of three such solutions when analyzed by the alpha-nitroso-beta-naphthol method (27) was found to be 2.00, 1.98, and 2.08 per cent of $CoCl_2$. This shows that hydrated cobalt chloride is very stable and thus an aqueous color solution can be prepared by weighing the hydrated crystals.

The next step involves the comparison of C-T curves of aqueous cobalt solutions containing varying amount of ethyl alcohol against the C-T curve obtained with aqueous $CoCl_2$.

For a summary, it can be stated that it is possible to determine 0.5 mg or more of barbital in simple solution, extraction of known quantities of barbital from tissues has resulted in 50 and 75 per cent recovery, and refinement of the above method by eliminating the turbidity may increase the accuracy of the test and per cent recovery from tissue.

THE EFFECT OF SODIUM BARBITAL INJECTED SUBCUTANEOUSLY TO MICE FED A 20% FAT DIET: This experiment begun in 1948 (28) was done to determine if a fatty liver was capable of detoxifying barbiturates.

One hundred eighteen normal six week old German strain mice were placed on a fat diet for eight weeks. One hundred grams of this high fat diet contained 20 grams of hydrogenated vegetable oil ("Snowdrift" or "Spry") and 80 grams of dehydrated food which is given in Table XVII.

Table XVII. Composition of Dehydrated Food

Ingredient	Quantity	Ingredient	Quantity
Wheat meal	30 lbs	Corn meal	20 lbs
Rolled oats	15 lbs	Dried cabbage	12 lbs
Dried whole milk ...	12 lbs	Dried whole eggs ..	6 lbs
Dried yeast	2 lbs	Calcium carbonate,	
Sodium chloride, ACS	1 lb	USP	1 lb
Cod liver oil	1.5 pints	Iron sulfate, USP .	0.25 lb

A control group consisting of 110 mice were given only the dehydrated food for the same period of time. Both groups were given water to drink ad libitum. At the end of the eight week experimental feeding period the control mice and the mice on the 20 per cent fat diet were injected with 0.10 or 0.15 ml of sodium barbital, each ml containing 111 mg of the barbiturate. The following data was recorded for both control mice and those on the fat diet used in this experiment.

1. Dietary Findings
   a. Weight at six weeks of age.
   b. Weight at 14 weeks of age.
2. Injection of Sodium Barbital
   a. Dosage, milligrams of sodium barbital per gram body weight.
   b. Death or recovery.
   c. Time (hours) required for death or recovery.

Table XVIII gives the results obtained with 14 week old mice injected subcutaneously with sodium barbital solution. As pointed out above, the mice were placed on a diet of water and (A) and (B) food at the age of six weeks.

Table XVIII. Toxic Effect of Sodium Barbital on Mice Fed a 20% Fat Diet

Av. Dose* (mg/g)	(A) 80% Dehydrated Food Plus 20% Fat		(B) 100% Dehydrated Food	
	Dead	Recovered	Dead	Recovered
0.62	8	6	6	3
0.67	13	10	14	10
0.72	18	10	19	10
0.77	25	8	17	11
0.82	19	1	15	5

* 0.62 represents 0.60-0.64; 0.67 represents 0.65-0.69, etc.

Results indicate that mice on a 20 per cent fat diet can resist barbiturate intoxication as well as the control group when injected with not more than 0.74 mg of sodium barbital per gram body weight. In the dose range between 0.60 and 0.74 the control group showed a 37 per cent (23 out of 62) recovery as compared to 39 per cent (26 out of 65) recovery for the mice on the fat diet. Increasing the dosage above 0.74 showed results which indicate that the mice on fat diet cannot resist barbiturate intoxication as well as the control group. In the dose range between 0.75 and 0.84 the control group showed a 33 per cent (16 out of 48) recovery in contrast to the 16 per cent (9 out of 53) recovery for the mice on a 20 per cent fat diet.

The procedure was repeated on two additional occasions to evaluate weight factors. The weight of every mouse in both the control and experimental group was recorded at the start of the eight week experimental feeding period and again on the day of sodium barbital injection. Table XIX gives the average weight of the mouse at six and fourteen weeks old. The average gain in weight is also tabulated.

Table XIX. Studies in Weight Gain on Mice Fed a 20% Fat Diet

Description	151 Mice on Fat Diet	148 Control Mice
Av. weight of mouse at 6 weeks (gram)	14.4	14.6
Av. weight of mouse at 14 weeks (gram)	21.3	21.3
Av. gain in weight (grams)	6.9	6.7

Table XIXa. Studies in Weight Gain on Male and Female Mice Fed a 20% Fat Diet

Description	Fat Diet		Control	
	74 Males	77 Females	74 Males	74 Females
Av. weight of mouse at 6 weeks (grams)	14.5	14.3	15.1	14.2
Av. weight of mouse at 14 weeks (grams)	22.0	20.7	22.0	20.6
Av. gain in weight	7.5	6.4	6.9	6.4

Results in Table XIX show that there is no difference in weight gain indicating that the mice on the 20 per cent fat diet consumed as much food as the

control group. Table XIXa shows that males gain a little more weight than the females during the eight week experimental feeding period. However, comparing females in the control group against females in the experimental group showed no difference in weight gain. The difference of 0.6 grams which occurred in the males is negligible.

Table XX shows that as the dosage is increased from 0.60 to 0.84 mg of sodium barbital per gram body weight the time required for death decreases in both the control group and those on a 20 per cent fat diet. Those mice which recovered in both groups require approximately the same number of hours to do so regardless of the dosage administered, the time for the control group to recover ranged from 25 to 34 hours as compared to 27 to 34 hours for the experimental mice.

Table XX. Average Time (hours) Required for Death or Recovery to Occur in Mice Injected with Sodium Barbital

Av. Dose* (mg/g)	Mice on Fat Diet		Control Mice	
	Death	Recovery	Death	Recovery
0.62	36	28	35	25
0.67	41	27	28	27
0.72	36	27	29	28
0.77	26	29	26	34
0.82	17	34	21	30

* 0.62 represents 0.60-0.64; 0.67 represents 0.65-0.69, etc.

Summary - 1. Mice on a 20 per cent fat diet can resist sodium barbital intoxication as well as the control group up to a dosage of 0.74 mg per gram body weight. Between 0.75 and 0.84 mg per gram body weight the control group gave 33 per cent recovery in contrast to 16 per cent for the mice on the 20 per cent fat diet. With such a paucity of data it is difficult to draw any conclusions. However, it appears that a fatty liver tends to diminish the detoxifying capacity of the liver when large amounts of sodium barbital are injected subcutaneously.

2. Both groups gained approximately the same weight during the eight week experimental feeding period, an average of 6.9 grams for the mice on the fat diet as compared to 6.7 gram for the control group. Males on a fat diet gained 7.5 grams as compared to 6.9 gram for the control group. The average gain in weight of the females was the same, namely 6.4 grams.

3. An increase in dosage results in a decreased time for death. However, recovery takes place in both groups in approximately the same time regardless of the dose administered.

THE EFFECT OF SODIUM BARBITAL INJECTED SUBCUTANEOUSLY TO MICE FED ON 8% ETHYL ALCOHOL: In 1948 it was reported (29) that mice on a 2 per cent alcoholic diet for a two weeks period reacted in the same manner as the control group when injected with varying concentrations of sodium barbital. It also was reported that mice fed on a 4 per cent alcoholic diet for a similar period showed an antagonistic reaction towards barbiturate intoxication. Using Trevan's procedure (30) the LD_{50} for alcoholic mice was found to be 0.65-0.69 (by Reed Muench formula (31) 0.67) in contrast to 0.55-0.59 (by Reed-Muench formula 0.59) mg per gram body weight for the control group. This study was continued in 1949.

A group of normal six week old German Bagg strain mice, subsequently referred to as alcoholic mice, were placed on an 8 per cent sugared ethyl alcohol

diet for a period of two weeks. This alcoholic beverage contained 2 grams of sucrose and 8 ml of dehydrated ethyl alcohol per 100 ml of aqueous solution. Another group (control mice) of the same strain was given tap water to drink in place of alcohol. A record was kept for 113 days to determine the amount of liquid consumed by each group. The average mouse colony per day was 43.6 and 41.9 mice for the alcoholic and control groups, respectively.

Table XXI gives the consumption of 8 per cent sugared ethyl alcohol solution and ordinary tap water. In addition, each group was subdivided into male and female mice.

Table XXI. Liquid Consumption of Mice

Description	8% Ethyl Alcohol	Tap Water
(A) Male and Female		
Total volume (ml) consumed	13,680	15,560
Volume (ml) consumed per day per colony	120.8	137.4
Volume (ml) consumed per day per mouse	2.8	3.3

(B) Male and Females separately

	Male	Female	Male	Female
Total volume (ml) consumed	6904	6562	8107	7178
Volume (ml) per mouse per day	2.8	2.8	3.3	3.1

Table XXI shows that the average daily consumption of alcoholic and control mice was 2.8 and 3.3 ml, respectively. The difference, 0.5 ml of liquid, between the two groups is not significant. What is important is that the mice do consume a solution of ethyl alcohol and in an amount comparable to those given water. The above table also shows that both male and female experimental mice consumed 2.8 ml of 8% alcohol in comparison to 3.3 and 3.1 ml for male and female control mice, respectively.

Both the alcoholic and control groups, in addition to being given either an 8 per cent ethyl alcohol solution or tap water, were fed for the two week experimental period the dehydrated food listed in Table XVII.

The weight of every mouse in both groups was recorded at the start of the two week feeding period and again 15 days later, i.e., on the day of the sodium barbital injection.

Table XXII gives the average weight of 345 alcoholic mice (165 males and 180 females) and 353 control mice (171 males and 182 females). The average gain in weight is also tabulated.

Table XXII. Studies in Weight Gain of 8% Alcoholic Mice

Description	345 Alcoholic Mice	353 Control Mice
(A) Male and Female		
Av. weight at 6 weeks (gram)	14.2	14.2
Av. weight at 8 weeks (gram)	17.4	17.8
Av. gain in weight	3.2	3.6

(B) Male and Female separately

	165 Males	180 Females	171 Males	182 Females
Av. weight at 6 weeks (grams)	14.4	14.0	14.6	13.9
Av. weight at 8 weeks (grams)	17.7	17.1	18.4	17.1
Av. gain in weight (grams)	3.3	3.1	3.8	3.2

Table XXII shows that the average weight gain for the alcoholic mice was 3.2 grams as compared to 3.6 grams for the control group. These values indicate that the alcoholic mice consumed as much food as the control group. The alcoholic males averaged 3.3 grams in weight gain in contrast to 3.8 grams for the control males. The average weight gained by the alcoholic and control females was 3.1 and 3.2 grams, respectively, indicating that both sexes consume approximately the same quantity of food during the two week period, i.e., from the sixth to the eighth week after birth.

Having established that alcoholic and control mice do consume comparable amounts of liquid and solid food, attention is now directed to the toxicity of subcutaneous injection of an aqueous solution of sodium barbital into both of these groups. Each group, consisting of 350 mice, were injected with varying volumes of sodium barbital, ranging from 0.05 to 0.15 ml. Each ml contained 111 mg of the barbiturate. The dosage varied from 0.50 up to and including 0.84 mg per gram body weight.

Table XXIII gives the results on 8 week old mice injected subcutaneously with sodium barbital solution. For convenience, all mice injected with 0.50 up to and including 0.54 mg/g are listed as receiving an average dosage of 0.52 mg/g, those injected with 0.55 up to including 0.59 mg/g are listed as receiving an average dosage of 0.57 mg/g, etc., subsequently referred to as a 0.52, 0.57 mg/g dosage level, etc. In each average dose listed there are 50 mice (25 males and 25 females). The numerical values represent those mice which recovered of the total injected.

Table XXIII. Toxic Effect of Sodium Barbital on Mice Fed 8% Alcohol

Av. Dosage* (mg/g)	Number of Mice Recovered					
	8% Sugared Ethyl Alcohol			Tap Water		
	25 injected		50 injected	25 injected		50 injected
	Male	Female	Total	Male	Female	Total
0.52	18	23	41	22	20	42
0.57	21	17	38	17	21	38
0.62	21	19	40	16	7	23
0.67	12	14	26	6	10	16
0.72	6	8	14	12	7	19
0.77	11	6	17	8	5	13
0.82	6	4	10	6	2	8

* 0.52 represents 0.50-0.54; 0.57 represents 0.55-0.59, etc.

Table XXIII shows that there is no difference in the mortality rate of either alcoholic or control group when from 0.50 to 0.59 mg/g of sodium barbital was injected subcutaneously. In these two dosage levels (0.52 and 0.57 mg/g) a total of 79 alcoholic mice recovered as compared to 80 in the control group. Forty and 23 of the 50 alcoholic and control mice, respectively, injected with 0.62 mg/g, recovered. This signifies that there were 34 per cent more recoveries in the alcoholic mice than in the control groups. In this dosage level there was an increase of 20% recovery. The last three dosages, namely, 0.72, 0.77 and 0.82 mg/g showed very little difference between the alcoholic and control groups. It should be mentioned, however, that the alcoholic mice injected with 0.72 mg/g had five less survivors than the corresponding control group. The total number of mice that recovered in the alcoholic group was equal to or greater than the number that recovered in the control group when given a corresponding dosage level with one exception, namely those injected with 0.72 mg/g. For all practical purposes the recovery of 41 and 42 mice in the alcoholic and control group, respectively, was considered equal.

Table XXIII also indicates that the LD_{50} (median lethal dose) seems to be between 0.65 and 0.69 mg/g for the alcoholic group in comparison to 0.60 and 0.64 mg/g for the control group. This can be demonstrated (Trevan's procedure) by combining any two ranges of dosages giving a total of 100 mice in each group, i.e.,

Alcoholic Mice		Control Mice	
0.60-0.69:	66 recovered	0.55-0.64:	61 recovered
0.65-0.74:	40 recovered	0.60-0.69:	39 recovered

Thus it appears from the available data that a two week diet which includes an 8% ethyl alcohol solution acts antagonistically to a subcutaneous injection of sodium barbital in any amount from 0.60 up to and including 0.69 mg/g; below 0.60 mg/g there appears to be no significant difference in the toxicity of the barbiturate; whereas no apparent difference, though inconclusive, was noted in dosages of 0.70 up to and including 0.84 mg/g.

Table XXIII also indicates that there is no significant difference in the mortality rate of either males or females in the alcoholic group. Four of the seven dosages listed showed a survival of more males than females, the remaining three dosage levels showed more females recovering than males. The maximum difference noted between recovered alcoholic males and females was 5 (11 males, 6 females injected with 0.77 mg/g). Out of a total of 350 alcoholic mice (175 of each sex) injected with sodium barbital 95 males and 91 females recovered. In the control group there were five dosage levels in which more males recovered than females with the greatest difference occurring at 0.62 mg/g in which 16 males and 7 females survived. Chi-square, using a four-fold table, was found to be 6.52 which indicates that a significant difference does exist. On the other hand, out of a total of 350 mice (175 of each sex) injected with sodium barbital, 87 males and 72 females recovered. In this case chi-square was found to be 2.59 which shows that no significant difference exists when all mice injected are considered.

The time required for either death or recovery to occur was used as another criteria to determine if any differences were noted between the alcoholic and control mice injected with sodium barbital. After the injection of the barbiturate, all mice were observed every two hours and the time of either death or recovery recorded. Table XXIV gives the average time, i.e., hours required for death or recovery to occur after the subcutaneous injection of sodium barbital into mice on either an alcoholic diet or tap water.

Table XXIV. Time (hours) Required for Death or Recovery to Occur in Mice Injected with Sodium Barbital

Av. Dosage* (mg/g)	8% Sugared Ethyl Alcohol Solution		Tap Water	
	Recovered	Dead	Recovered	Dead
0.52	18	15	18	36
0.57	27	37	21	30
0.62	26	30	22	31
0.67	26	30	21	34
0.72	30	26	24	35
0.77	29	21	28	18
0.82	22	18	29	22

* Av. Dose: 0.52 represents 0.50-0.54; 0.57 represents 0.55-0.59, etc.

It is difficult to make any generalizations from the data given in Table XXIV. Nevertheless, in both the alcoholic and control groups, it took slightly longer for mice to die than to recover when 0.57, 0.62, and 0.67 mg. of sodium barbital per gram body weight was administered subcutaneously. When 0.77 and 0.82 mg/g of

barbiturate was injected, the reverse was noted, i.e., the time necessary for recovery was longer than that required for death. It appears that if recovery does not occur in 21 or 26 hours in those control and alcoholic mice, respectively, injected with either 0.57 or 0.62, or 0.67 mg/g then death will more than likely occur at a subsequent time. On the other hand, if death does not occur within approximately 20 hours in those alcoholic and control mice injected with 0.77 or 0.82 mg/g then recovery of these mice is highly probable.

With the exception of the average dose of 0.82 mg/g it takes approximately the same time or longer for alcoholic mice to recover than it did for the control group. As an example, alcoholic mice injected with 0.57 and 0.62 mg/g required 27 and 26 hours, respectively, for recovery in comparison to 21 and 22 hours for the control group. It is interesting at this point to mention that although there is an antagonistic action of alcohol toward barbital, yet it takes the alcoholic mice longer to recover than the controls. A possible explanation to this phenomenon may be that after 21 hours in the case of control mice injectected with 0.62 mg/g the possibility of death becomes increasingly greater with an increase in time. The alcoholic mice, on the other hand, recover in 26 hours, signifying that the body has five additional hours to detoxify the barbiturate before an increase in time will cause death.

It was decided at the beginning of the experiment that all alcoholic and control mice injected with 0.10 ml (11.1 mg) of sodium barbital be observed until they had become completely anesthetized, i.e., comatose, with the complete absence of convulsions. A description of the action of the subcutaneous injection of sodium barbital follows: Shortly after sodium barbital was administered all mice walked with a staggering gait which became progressively worse and was followed by the inability to walk at all. Then in five or ten minutes after the injection there was a short period of convulsions which lasted between 15 minutes to an hour or longer, depending on the amount of barbiturate injected, and finally the mouse became comatose. Almost immediately after the subcutaneous administration of the barbiturate the mouse started to scratch the site of the injection.

Table XXV which follows gives the average time (minutes) required for alcoholic and control mice to become completely anesthetized when 0.10 ml (11.1 mg) of sodium barbital was injected subcutaneously.

Table XXV. Time (minutes) Required to Anesthetize Completely Mice with Sodium Barbital

Description	Av. Dose* (mg/g)						
	0.52	0.57	0.62	0.67	0.72	0.77	0.82
(A) All mice							
Alcoholic	55	43	43	39	36	33	24
Control	56	47	41	40	37	32	29
(B) Males							
Alcoholic	52	41	38	35	39	32	22
Control	56	42	42	35	40	26	25
(C) Females							
Alcoholic	58	45	47	42	35	34	25
Control	57	42	41	43	36	36	31

* Av. dose: 0.52 represents 0-50-0.54; 0.57 represents 0.55-0.59, etc.

Table XXV shows the expected trend: As the dosage is increased the time for complete anesthesia of the mouse is decreased. The average maximum time required to anesthetize a mouse injected with 0.52 mg/g of sodium barbital was 55 and 56 minutes for alcoholic and control mice respectively. Those alcoholic and control mice injected with the highest dosage, 0.82 mg/g, became completely anesthetized in 24 and 29 minutes, respectively. No significant differences in the time to anesthetize a mouse were noted in any of the dosages administered between the following: alcoholic and control groups, alcoholic and control males, alcoholic and control females. A slight difference, however, was noted between the time required to anesthetize males and females in the alcoholic as well as in the control group. Table XXV shows that in 11 of the 14 possible male and female groups, the latter required more time to become anesthetized than the corresponding former group. The three exceptions where males required more time to become anesthetized were: one in the alcoholic group (0.72 mg/g) and two in the control group (0.62 and 0.72 mg/g).

The percentage gain (or loss) in weight for the alcoholic and control group was calculated for the two week experimental feeding period in order to determine if any correlation existed between an increase (or decrease) in body weight and the occurrence of recovery or death subsequent to the subcutaneous injection of sodium barbital. All males and females were segregated at 4 weeks of age to eliminate any increase in weight of the females due to pregnancy. No more than 5 mice were put in one cage.

Table XXVI gives the minimum and maximum percentage weight gain (or loss) of alcoholic and control mice on a two week diet of dehydrated food and an 8 per cent sugared ethyl alcohol solution or tap water.

Table XXVI. Maximum and Minimum Per Cent Weight Gain (or Loss) of Mice from Six to Eight Weeks of Age

Av. Dose* mg/g	Sex	Effect	% Gain (or Loss) in Weight Alcoholic	Control
0.52	Male	Recovered	6 to 57	0 to 61
	Male	Dead	1 to 123	17 to 32
	Female	Recovered	6 to 54	-2 to 55
	Female	Dead	37 to 53	1 to 50
0.57	Male	Recovered	2 to 70	-5 to 64
	Male	Dead	-45 to 21	4 to 58
	Female	Recovered	-3 to 48	8 to 97
	Female	Dead	6 to 40	16 to 98
0.62	Male	Recovered	7 to 52	-6 to 101
	Male	Dead	14 to 60	10 to 74
	Female	Recovered	0 to 62	-18 to 62
	Female	Dead	6 to 67	0 to 63
0.67	Male	Recovered	4 to 61	-18 to 32
	Male	Dead	-7 to 45	2 to 66
	Female	Recovered	1 to 41	10 to 57
	Female	Dead	10 to 62	2 to 94
0.72	Male	Recovered	5 to 56	6 to 55
	Male	Dead	-26 to 56	-4 to 90
	Female	Recovered	2 to 42	3 to 50
	Female	Dead	0 to 80	-54 to 57
0.77	Male	Recovered	8 to 48	19 to 37
	Male	Dead	-7 to 54	7 to 46
	Female	Recovered	10 to 31	3 to 35
	Female	Dead	11 to 67	-6 to 44
0.82	Male	Recovered	7 to 37	11 to 100
	Male	Dead	-9 to 80	0 to 102
	Female	Recovered	14 to 48	13 to 26
	Female	Dead	-8 to 63	21 to 92

* Av. Dose: 0.52 represents 0.50-0.54; 0.57 represents 0.55-0.59, etc.

Table XXVI shows that the minimum or maximum percentage gain (or loss) in weight does not have any relationship to either death or recovery resulting from a subcutaneous injection of sodium barbital. This is important because some mice which are not as healthy as others can recover from barbiturate intoxication. This is further demonstrated by a loss of weight by several mice during the two week experimental feeding period which subsequently recovered from an injection of sodium barbital. These mice were: Alcoholic ..... female given 0.57 mg/g; control ..... female given 0.52 mg/g, male given 0.57 mg/g, male and female given 0.62 mg/g, and male given 0.67 mg/g.

The following summary can be made on the basis of the work performed on 350 mice fed dehydrated food and an 8 per cent sugared ethyl alcohol solution and an additional 350 mice given the same dehydrated food but water instead of alcohol.

Summary: 1. Alcoholic mice consumed an average of 2.8 ml of 8 per cent ethyl alcohol solution which is comparable to the 3.3 ml of water consumed by the control mice.

2. Alcoholic mice consumed as much food as the control mice, the former averaged a gain of 3.2 grams for the two weeks experimental period in comparison to 3.6 grams for the control group.

3. The continual use of 8% ethyl alcohol solution as a beverage for a two week period appears to have some protective action against sodium barbital intoxication. The LD50 for alcoholic mice was 0.65-0.69 as compared to 0.60-0.64 mg/g of sodium barbital for the control group.

4. Usually more time (hours) was required for recovery after barbiturate administration for alcoholic mice than in control mice injected with the same amount of sodium barbital.

5. An increase in dosage resulted in a decreased time (minutes) to completely anesthetize the mouse. No difference was noted between alcoholic or control group.

6. Percentage gain in weight for the two week experimental feeding period does not influence the occurrence of death or recovery in either group.

7. Average liquid consumption, gain in weight, time required for recovery due to barbiturate intoxication, and the toxicity of sodium barbital, does not differ significantly between males and females. It appears that females require longer to become anesthetized than males.

BACTERIOLOGY SECTION

The section is composed of the following functional sub-divisions:

1. Diagnostic Medical Bacteriology
2. Bacteriology of water, ice, and milk products
3. Enteric Bacteriology
4. T. B. Diagnosis
5. Mycology
6. Bioassay
7. Biologics production
8. Media preparation
9. Animal room
10. Central Supply

Routine

During 1949 routine procedures amounted to 46,293, representing an 82% increase over that for the preceding year. This is in part a reflection of a determined effort on the part of members of the section to develop better liaison with, and service to, other laboratories. A particularly marked increase was in bacteriological examination of water following establishment of more control and interest in this field of sanitation. At the close of the year almost 2000 specimens of this type are received monthly.

Routine work will not be presented as such. Special projects incident to training of changing personnel and attempts at improvement of laboratory service offered give some indication of the scope of the basic work performed.

Research

Streptolysin Production - The determination of optimal concentration of various ingredients in media for greater streptolysin O production by Streptococcus pyogenes (strains H-46-A, C-203-M and C-203-S) have been investigated. The following basal medium was employed:

Bacto-veal	5.0 g.
Proteose-peptone	2.0 g.
NaCl	0.2 g.
Na_2HPO_4	0.1 g.
Dextrose	0.2 g.
Double distilled water q.s.	100 cc.

The Bacto-veal powder is infused in less than 100 cc. distilled water at 50°C. Add peptone, NaCl, Na_2HPO_4 and dextrose, heating gently to dissolve. Adjust pH to 7.6, refilter and autoclave at 15 lbs for 15 minutes. To the above base, add aseptically $NaHCO_3$ sterilized through Seitz filter to a final concentration of 0.25% and add maltose solution to a final concentration of M/200. Considering maltose, dextrose, Na_2HPO_4 and $NaHCO_3$ as four important factors, the experiment was carried out by changing the concentration of one factor with fixed concentrations of the other three factors specified in the formula. The results are presented in Charts I, II and III (Figure 1). From these results it may be stated that strain C-203-M is a poor producer

Figure 1

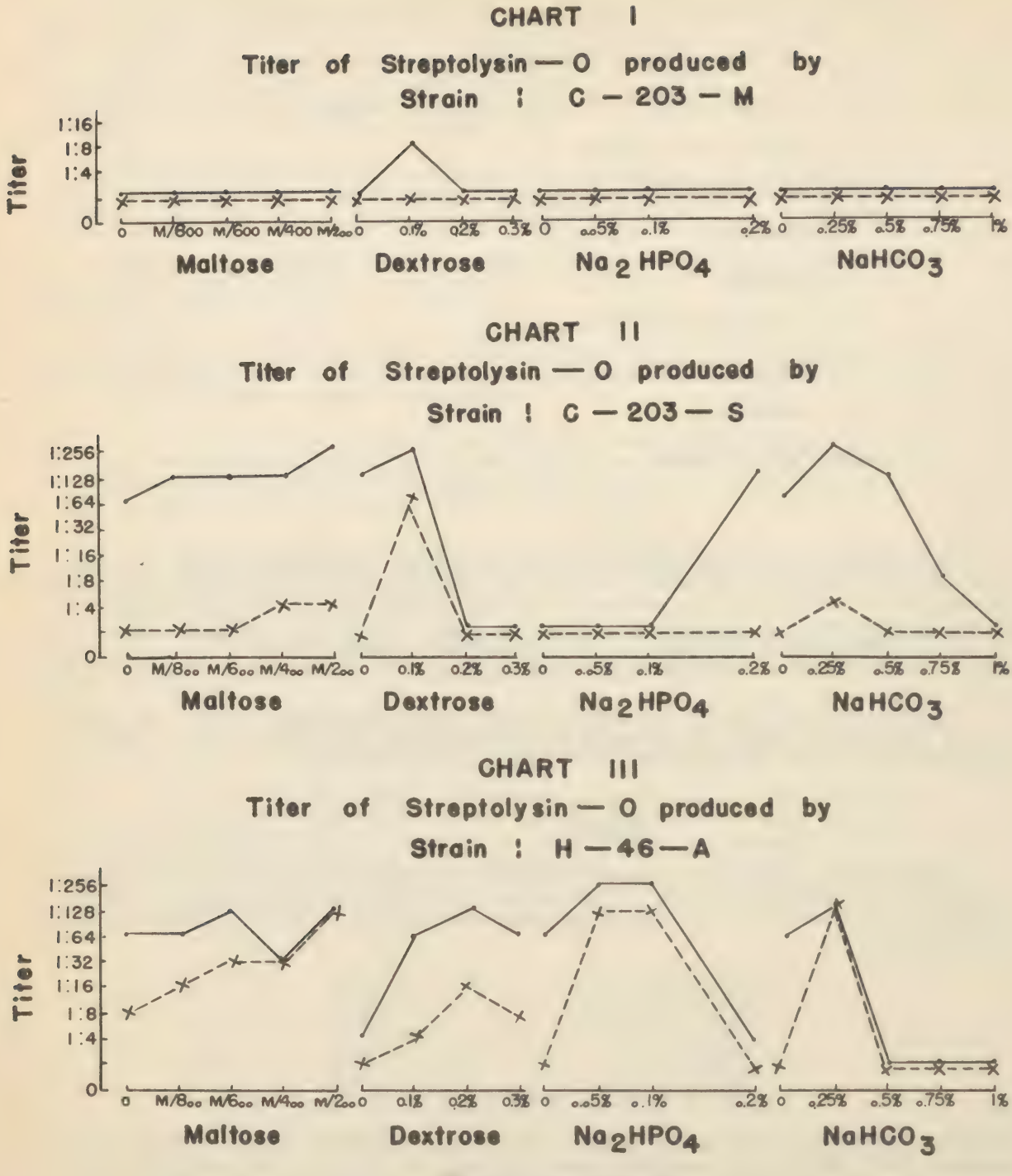


CHART I
Titer of Streptolysin — O produced by
Strain ! C — 203 — M
Titer
1:16
1:8
1:4
0
M/800 M/600 M/400 M/200
0.1% 0.2% 0.3%
.5% .1% .2%
.25% .5% .75% 1%
Maltose
Dextrose
Na2HPO4
NaHCO3
CHART II
Titer of Streptolysin — O produced by
Strain ! C — 203 — S
Titer
1:256
1:128
1:64
1:32
1:16
1:8
1:4
0
M/800 M/600 M/400 M/200
0.1% 0.2% 0.3%
.5% .1% .2%
.25% .5% .75% 1%
Maltose
Dextrose
Na2HPO4
NaHCO3
CHART III
Titer of Streptolysin — O produced by
Strain ! H — 46 — A
Titer
1:256
1:128
1:64
1:32
1:16
1:8
1:4
0
M/800 M/600 M/400 M/200
0.1% 0.2% 0.3%
.5% .1% .2%
.25% .5% .75% 1%
Maltose
Dextrose
Na2HPO4
NaHCO3
Fixed concentration
reduced
unreduced
Maltose M/200
Na2HPO4 0.1%
Dextrose 0.2 %
NaHCO3 0.25 %

of streptolysin O. Strain C-203-S produces high yield with:

M/200	Maltose
0.1%	Dextrose
0.2%	Na_2HPO_4
0.25%	$NaHCO_3$

The highest yield of streptolysin O was obtained with strain H-46-A when the ingredients of the basal medium supply are:

M/600	Maltose
0.2%	Dextrose
0.05%	Na_2HPO_4
0.25%	$NaHCO_3$

A Holding Medium and Method for N. gonorrhea - Mention has been made previously (1) of studies begun in 1948 relative to the problem of diagnosis of gonorrhea by cultural methods. A suitable method of maintaining suspected inocula for subsequent culture under optimal conditions is a common desire in area laboratory operations (2). The method must allow the central laboratory to supply special media in such a form as to permit satisfactory storage and shipment. The use of a standard 2 oz. screw cap prescription bottle (4-060-150) containing a one quarter inch layer of solid media lengthwise of the bottle, satisfies this requirement. The nature of the container not only allows ease of shipment but also retains moisture for long periods of time and affords apparently a slightly reduced oxygen tension desirable for growth of N. gonorrhea.

Comparative studies were conducted in 1948 on 100 females clinically considered to have active gonorrhea. Material obtained with sterile swabs from cervical discharges were streaked on each of two media (chocolate agar and blood agar) in petri dish and screw cap vial. One hour incubation under reduced oxygen tension revealed 82% recovery from the screw cap containers (Table I), indicative of the reduced area plated. Using the same two media and comparing recovery with immediate incubation and following the 24 hour delay comparable to that occasioned by shipment from other laboratories, the degree of recovery again appears worthwhile (Table II). Delayed incubation resulted in 29 and 37% less with media 1 and 2 respectively. Parallel study with a third medium (chocolate agar with yeast extract) shows markedly better recovery from the original 100 cases, and only 14% less with delayed incubation.

Table I. Comparison of petri dish and screw cap vial

No. of Suspects	Positive Cultures Obtained with Optimal Conditions			
	Petri Dish Controls		Screw Cap Vials	
100	Medium #1	Medium #2	Medium #1	Medium #2
	No.	No.	No. %	No. %
	58	56	49 84.48	46 79.30

Table II. Recovery of N. gonorrhea with delayed incubation

	Immediate (1 hour) incubation	Delayed (24 hour incubation)
Medium #1	49	35 (71%)
Medium #2	46	29 (63%)
Medium #3	51	44 (86%)

In view of a recent publication concerning selective media for culture of N. gonorrhea (3) second comparative study was performed using Carpenter's medium No. II. and our own Medium No. 3 above. The composition of these media is as follows: (Table III).

Table III. Composition of Test Media

Ingredients	Test Medium No. 3	Carpenter's Medium II
Proteose peptone No. 3 Difco	X	X
Citrated human blood 7.5%/v	X	
Yeast extract 10%/v	X	
Supplement B, Difco		X
Haemoglobin 2.0% sol., Difco		X
Corn starch		X
Bacto-agar	X	X
Dextrose	X	X
NaCl	X	X
Na_2HPO_4	X	
K_2HPO		X
KH_2PO_4		X

As stated above, since our primary need is for a medium suitable for maintenance and shipment, the comparison included both test media in screw cap vials with delayed incubation under optimal conditions. The factor of storage and delay in use of media has not yet been considered. As a control means of determining the number of cases in which recovery by cultural methods might be expected our medium No. 3 was used in petri dishes with optimal cultural conditions. Recovery from cervical swabs obtained from 100 additional females shows an almost identical rate of recovery with both test media (Table IV).

Table IV. Recovery of N. gonorrhea on Various Holding Media

Number of Specimens	Incubation (direct)					
From females 1949	Cultures positive on control media		Cultures positive on Carpenter's Medium II		Cultures positive on 406th Medium No. 3	
	No.	%	No.	%	No.	%
100	81	81	50	62	51	63

These preliminary studies warrant further more exhaustive comparison between various media. Such work is now in progress.

Variation of a Shigella Serotype In Vivo - Variation within the many serotypes of the genus Shigella has been observed and described on numerous occasions (Takita, (4), Boyd (5) and Veazie (6). The exact nature of these changes is still a matter for argument although Boyd's idea of a loss variation seems to explain best the majority of such phenomena. In so far as we can determine, such variations have resulted from old stock cultures or from parent cultures which threw off variants, usually in two directions. We have recently, however, observed this loss variation in a long standing case of human dysentery.

On 12 September 1949 a stool culture was submitted from a patient by the 128th Station Hospital. Over a period of about six weeks, during which time the man was hospitalized, twelve stool specimens were submitted. From the first five of these a pathogen was isolated and identified as S. paradysenteriae type W. From the following six specimens an organism was obtained which reacted in a manner different from the originally isolated organisms. From the twelfth stool no enteric pathogen was obtained.

Comparative agglutination studies proved that the first organism isolated (W-A) was specific in its reactions, there being little evidence of cross-agglutination relationships with other Flexner strains. On the other hand the second type W-B, showed a good mosaic of group antigens, reacting with the majority of the Flexner types in an avid fashion. It was further demonstrated by reciprocal absorption experiments conducted with antisera prepared against the two types that W-A actually was richer in specific substance, while W-B had lost much of this antigen and had either gained or had unmasked a number of group antigens. Preliminary experiments indicate that the unmasking hypothesis probably prevails. This has an exact analogy in Body's work with the 103A-103B variation.

In view of this shift in antigenicity in vivo we believe that further work is indicated to complete the immunological work and that the case is unique enough to report in a scientific journal. This will be accomplished in the near future.

Coproantibody Studies - Studies described previously (4) were continued for a total of 882 additional stool specimens processed during 1949. Overall review of the results do not warrant continuation of the project.

The Isolation of Three Serotypes of S. Paradysenteriae from a Single Case - On 8 December 1949 plates of S.S. and EMB agar containing non-lactose fermenting colonies were received from the 155th Station Hospital. Four colonies were picked from the S.S. agar, two from the EMB to Kligler (1% sucrose added) slopes. Five proved to have the presumptive biochemical characteristics of the genus Shigella: all agglutinated in Shigella group A sera. The use of specific typing sera proved one to be type 103, two were VZ and two Boyd 88.

All organisms were purified by restreaking and the biochemical and serological properties checked. The types were verified and biochemical tests found consistent. Of some interest was the fact that those organisms typed as Boyd 88 were gas producers in both dextrose and mannitol: thus Manchester strains.

A repeat stool specimen nine days later found all selected colonies to be Type VZ. Isolation, however, of the Manchester strain was also accomplished by the 155th Dispensary.

Phase Variation and S-B Variation in Shigella Dysenteriae - Benians (7) in 1919 described a variation in "Shiga's dysentery bacillus" which agrees generally with the observations set down by Arkwright (8) in 1921 as S-R variation for "B. dysenteriae Shiga." Benian's specific-serum agglutinable strain produced diffuse growth in broth and stable suspensions in 0.85% NaCl solution, whereas the inagglutinable strain tended to form a sediment in broth and to be unstable in saline. Both strains proved fatal for rabbits. Vaccines of either strain afforded protection when vaccinated animals received challenges either with the homologous or heterologous strain. In establishing the existence of the two distinct Shiga toxins suggested by Olitski and Kligler in 1920, Boivin (9) pointed out that S-variants produce both an endotoxin (enterotoxin) and exotoxin (neurotoxin) whereas B-variants produced only an exotoxin. Haas (10) was in agreement with these correlations between S and R variation and differences in toxin production. It is evident from the foregoing that most workers dealing with variations in Shiga's bacillus have concerned themselves with that variation in relation to toxin production. The purpose of the present report is to consider certain serologic and biochemical aspects of a variation commonly observed in Shiga's bacillus and to suggest that such variation is not, in effect, merely a dissociation from S to R.

Strains of Shigella dysenteriae employed included two of a number isolated in Japan, three strains received from William Ewing, Enteric Bacteriology Laboratories, Chamblee, Georgia and one standard strain, 43-A-1, from the U. S. Army Medical Department Research and Graduate School. Of the strains from Ewing, only one, EW-83, after repeated subcultures, produced variants and those were not identical with the variation observed in freshly isolated strains. Strain 43-A-1, though in use here for several years, was not observed to give rise to variants of the type to be reported. Of the locally isolated S. dysenteriae strains 136 and 167 will be discussed in detail in this report.

General

When subcultures from fresh isolates of S. dysenteriae were studied on tryptose agar, it was observed that three types of even contoured colonies occurred: clear (translucent), opaque, and clear-opaque. The line of demarcation between the clear portion and the opaque portion in the latter type of colony was often median and the contrast in the amount of light transmitted by each of the two halves was quite marked. Tryptone broth cultures of all three types grew as even suspensions, and saline suspensions of cells of each type, harvested from agar slopes, were stable.

The end types, T (clear) and O (opaque), could be distinguished by two means, one serological and the other biochemical. The O cells were agglutinated by absorbed, specific, diagnostic S. sonnei, phase II, antisera using slide technic and were capable of effecting the hydrolysis of maltose. T cells were incapable of hydrolyzing maltose and were inagglutinable in the presence of S. sonnei, phase II, antiserum. Populations of cells derived from T-O colonies hydrolyzed maltose but were agglutinable by S. sonnei, phase II., absorbed, specific sera. The direction of variation was from T to O and in the exceptional cases where T cells were derived from subcultures O colonies it was assumed that such O colonies were imperceptibly contaminated with T cells. With great care and continuous subculture it was possible to obtain T-cultures with little or no tendency towards dissociations and O-cultures which also remained biochemically and serologically stable. This tendency of old stock T strains to become stable, as has already been mentioned above, was borne out by the behavior of AMDR&GS strain 43-A-1 and by three of the four strains received from Ewing.

Validity of the Strains Isolated in Japan - The common identity of the strains isolated in this laboratory and those received from other sources was easily established by serological and biochemical means. Cells derived from clear colonies of all strains investigated were agglutinated by S. dysenteriae antiserum prepared by AMDR&GS and S. dysenteriae, EW-83 antiserum (Lot No. 500) received from Dr. Ewing. Reciprocal absorption between antigen-antibody systems derived from 136-T, 167-T and from EW-83 served to further serologically confirm the identity of those strains. The T-type cells from all strains uniformly produced acid from dextrose and trahalose only.

Phase Variation - Wheeler and Mickle pointed out that the variation generally regarded as smooth-to-rough variation in Shigella sonnei was in effect an irreversible phase variation and they designated the phases as I and II. They were able to show that from phase II, under appropriate conditions there could be derived a true rough S. sonnei variant. A similar series of phenomena seems to be involved in the dissociation of recently isolated Shigella dysenteriae. In keeping with Wheeler's phase designations, the T-colonies, already described, should probably be termed S. dysenteriae, phase I, and the O colonies as S. dysenteriae, phase II. The place of this phase variation in the formation of rough variants will be discussed below.

Strain 136 - Strain 136 was isolated in November 1948 from an acute case of bacillary dysentery in which serum and fecal agglutinins for 136 phase I (T) and 136 phase II (O) were demonstrated. Sera were prepared against each phase, separately in rabbits. Phase I sera failed to agglutinate phase II antigen; phase II sera in high concentrations, however, agglutinated cells of phase I. No cross agglutination occurred between phases when the slide agglutination technique was employed and this was the method routinely used for serologic checking. The ability of these sera to agglutinate other Shigella species is shown in Figs. 2, 3, 4. Strain 136 was maintained in two ways: (1) both

Figure 2

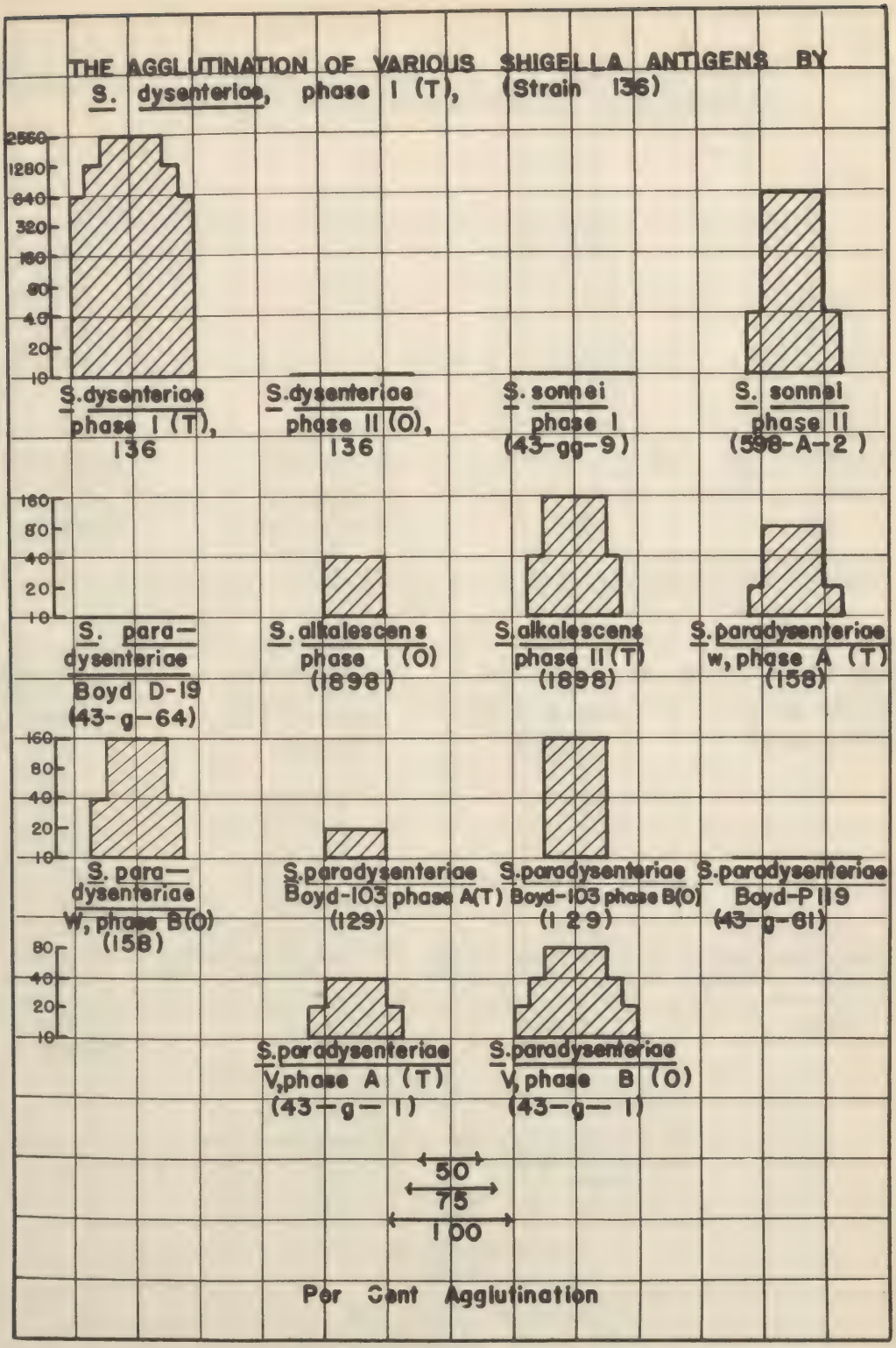

THE AGGLUTINATION OF VARIOUS SHIGELLA ANTIGENS BY S. dysenteriae, phase I (T), (Strain 136)
2560
1280
640
320
160
80
40
20
10
S. dysenteriae phase I (T), 136
S. dysenteriae phase II (O), 136
S. sonnei phase I (43-gg-9)
S. sonnei phase II (598-A-2)
160
80
40
20
10
S. para-dysenteriae Boyd D-19 (43-g-64)
S. alkalescens phase I (O) (1898)
S. alkalescens phase II (T) (1898)
S. paradysenteriae w, phase A (T) (158)
160
80
40
20
10
S. para-dysenteriae W, phase B(O) (158)
S. paradysenteriae Boyd-103 phase A(T) (129)
S. paradysenteriae Boyd-103 phase B(O) (129)
S. paradysenteriae Boyd-P119 (43-g-61)
80
40
20
10
S. paradysenteriae V, phase A (T) (43-g-1)
S. paradysenteriae V, phase B (O) (43-g-1)
50
75
100
Per Cent Agglutination

Figure 3

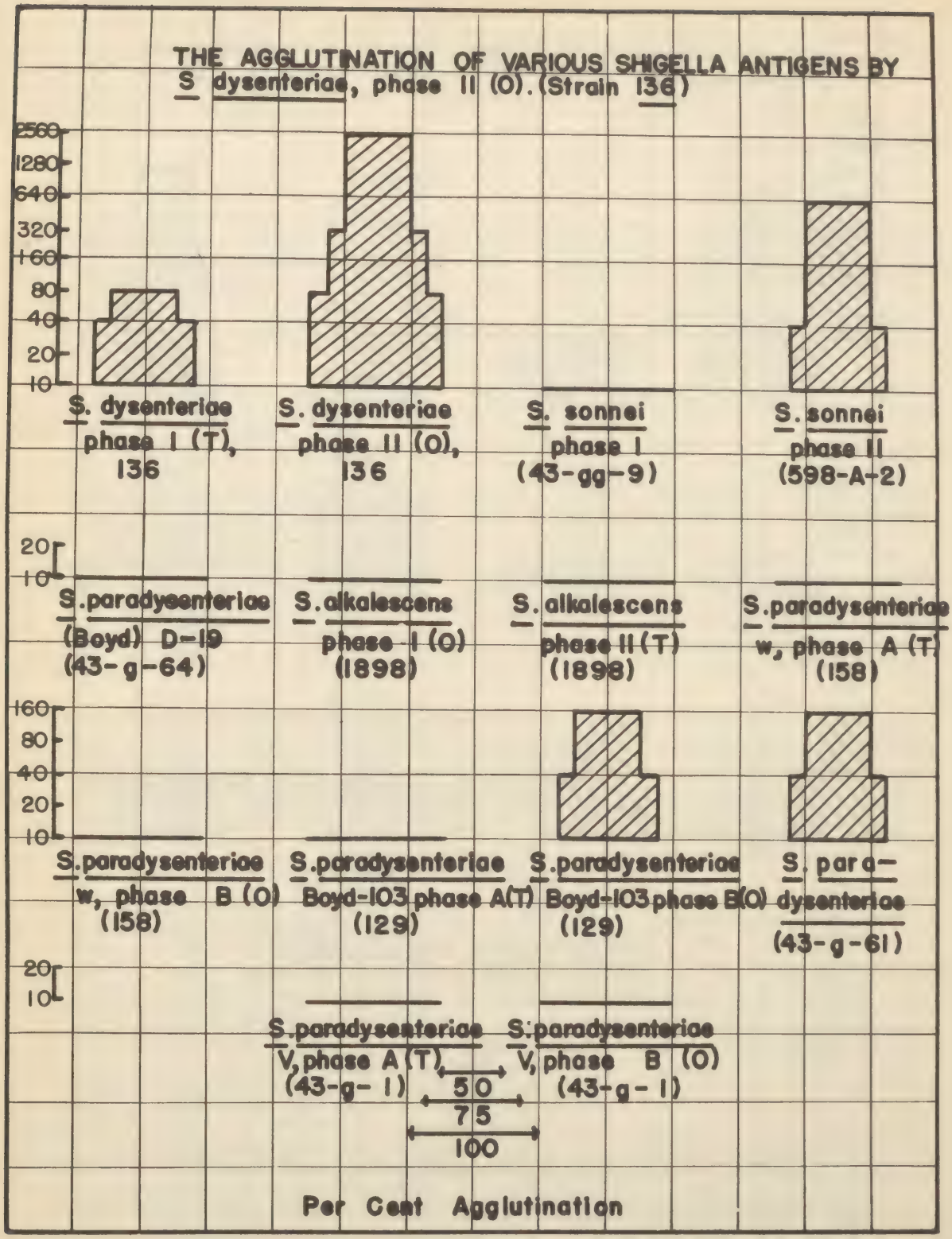
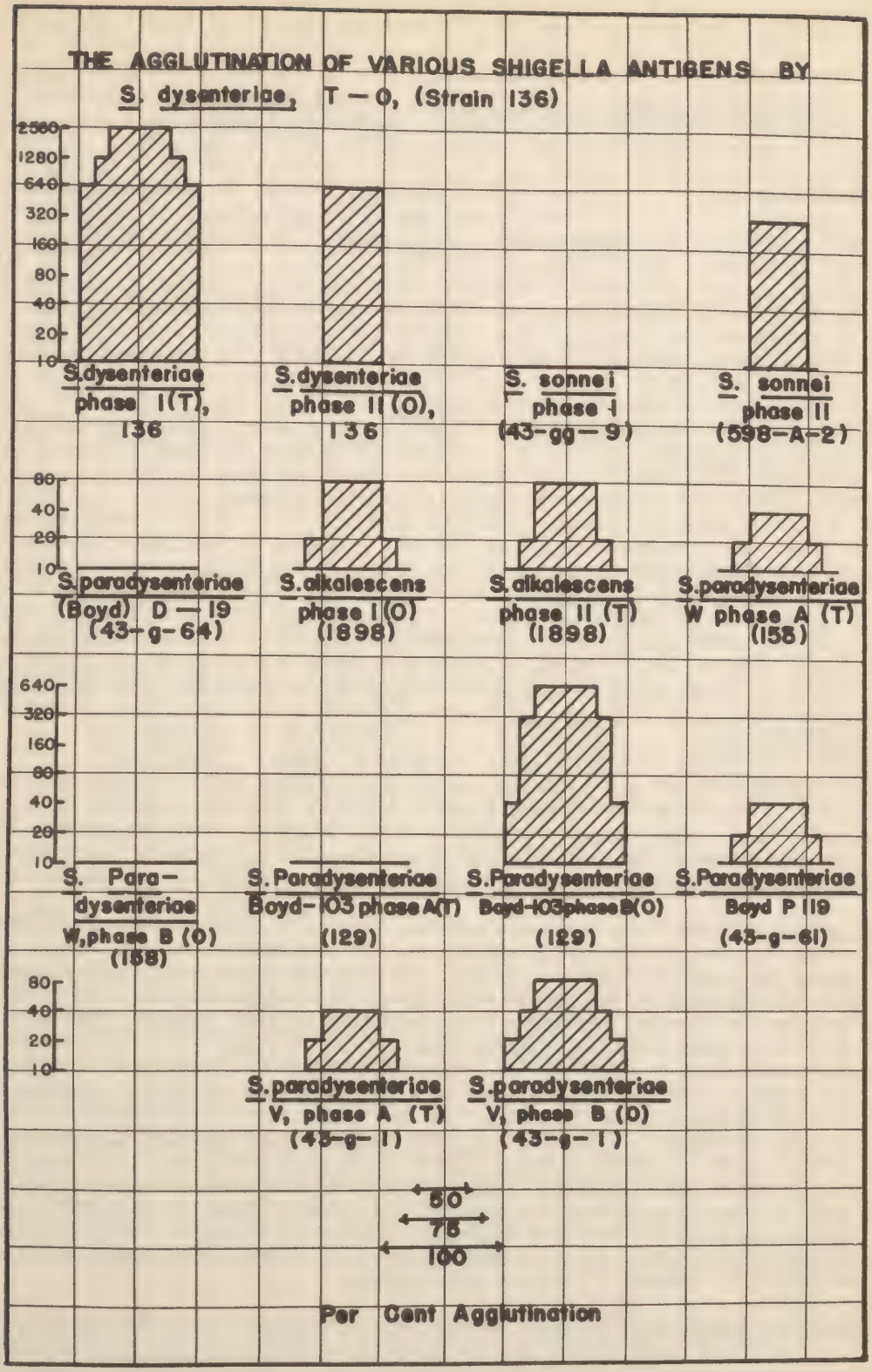

THE AGGLUTINATION OF VARIOUS SHIGELLA ANTIGENS BY
S dysenteriae, phase II (O). (Strain 136)
2560
1280
640
320
160
80
40
20
10
S. dysenteriae phase I (T), 136
S. dysenteriae phase II (O), 136
S. sonnei phase I (43-gg-9)
S. sonnei phase II (598-A-2)
20
10
S. paradysenteriae (Boyd) D-19 (43-g-64)
S. alkalescens phase I (O) (1898)
S. alkalescens phase II (T) (1898)
S. paradysenteriae w, phase A (T) (158)
160
80
40
20
10
S. paradysenteriae w, phase B (O) (158)
S. paradysenteriae Boyd-103 phase A(T) (129)
S. paradysenteriae Boyd-103 phase B(O) (129)
S. para-dysenteriae (43-g-61)
20
10
S. paradysenteriae V, phase A (T), (43-g-1)
S. paradysenteriae V, phase B (O) (43-g-1)
50
75
100
Per Cent Agglutination

Figure 4


THE AGGLUTINATION OF VARIOUS SHIGELLA ANTIGENS BY S. dysenteriae, T — 0, (Strain 136)
2560
1280
640
320
160
80
40
20
10
S.dysenteriae phase I(T), 136
S.dysenteriae phase II (O), 136
S. sonnei phase I (43-gg — 9)
S. sonnei phase II (598-A-2)
80
40
20
10
S.paradysenteriae (Boyd) D — 19 (43-g-64)
S.alkalescens phase I (O) (1898)
S.alkalescens phase II (T) (1898)
S.paradysenteriae W phase A (T) (158)
640
320
160
80
40
20
10
S. Para-dysenteriae W, phase B (O) (158)
S. Paradysenteriae Boyd-103 phase A(T) (129)
S.Paradysenteriae Boyd-103phase B(O) (129)
S.Paradysenteriae Boyd P 119 (43-g-61)
80
40
20
10
S.paradysenteriae V, phase A (T) (43-g-1)
S.paradysenteriae V, phase B (O) (43-g-1)
50
75
100
Per Cent Agglutination

phases were preserved in the dry state in evacuated, sealed tubes and (2) both phases were carried on tryptose agar and periodically replated and colonies repicked. For a period of nine months phase I (T) remained serologically and biochemically as it had appeared upon initial isolation. Phase II (O), however, underwent marked changes. For the first five months or so cells derived from this phase exhibited no detectable serological or biochemically as it had appeared upon initial isolation. Phase II (O), however, underwent marked changes. For the first five months or so cells derived from this phase exhibited no detectable serological or biochemical alteration, though a concerted effort was made to find colonies which might be rough in character. It was observed, however, that as phase II (O) was continuously subcultured its ability to grow as an even suspension in tryptone broth diminished. Cell suspensions prepared at this time and heated to 100 C for five minutes were autoagglutinable in saline, a characteristic not manifested by either phase I (T) or phase II (O) within the first sixty days following isolation. Also, the length of time required for II (O) cells to effect the hydrolysis of maltose increased from 48 hours to 120 hours. By daily plating and picking of these phase II subcultures which did not grow as even suspensions in broth, colonies with irregular margins were noted. These were sub-cultured and designated as 136-II-R-1, R-2 and R-3. R1 and R-3 were colonially similar with irregular margins, were of larger size than their parent, 136-II (O), and their surfaces were moist in appearance. R-2 was smaller in size and its surface was rugose and appeared dry. All three, R-1, R-2 and R-3, grew as a heavy sediment in tryptone broth in contrast to the cloudy growth of their parent, 136-II (O). All differed from the parent type in being able to produce acid from dextrin. All exhibited slight instability in saline and were agglutinated by 136-II (O) serum and S. sonnei, phase II antiserum (Fig. 5). When suspensions of R-1 cells were titrated in parallel with 136-II (O) suspensions against phase II antisera, the difference in titer was only a matter of one tube. In view of the fact that some spontaneous agglutination occurred in the saline control tube of 136-R-1 and that the optimal antigen-antibody proportions for it were unknown, it was felt that a serological difference could not be demonstrated merely on the basis of a parallel titration. When phase II (O) serum was once absorbed with 136-R cells, however, a definite lowering in the specificity of the serum for 136-R cells, without a similar lowering of its specificity for phase II (O) cells occurred. This difference was most marked when the antigens employed were prepared by heating in alcohol at 65°C for one hour (Fig. 6).

Strain 167 - Strain 167 was isolated subsequently from a case of acute bacillary dysentery. Subcultures from 167 yielded T and O colonies indistinguishable from those described above for S. dysenteriae strain 136. Phase specific 167-I (T) and 167-II (O) antisera functioned interchangeably with 136 phase specific antisera in the serological differentiation of phase antigen. A rough variant was not produced from strain 167.

Strain EW-83 - As has been mentioned earlier, of the strains received from Ewing only EW-83 could be induced to undergo variation. This variation was not comparable to that observed in freshly isolated strains. It involved three distinct colonies: one clear with entire margins, one clear with irregular margins and one which was irregular in outline and opaque. Antigens prepared from the even colonies reacted only with phase I sera (prepared with S. dysenteriae, 136-I: antigens from the clear colonies with irregular margins agglutinated strongly with phase I serum and to about 33% of titer with phase II serum. Neither the cells from the even contoured colonies nor those from the irregular colonies brought about the hydrolysis of carbohydrates other than dextrose and trehalose. Cells derived from the irregular margined opaque colonies reacted only with phase II serum of the S. dysenteriae sera used, and hydrolyzed maltose in addition to dextrose and trehalose. These same cells from the irregular opaque colonies were agglutinated by low concentrations of S. sonnei, phase II, antiserum (see Fig. 5). Of the antigens derived from these three types of colonies, only that from the irregular-margined, opaque colonies was unstable in saline. The opaque and irregular-contoured colonies are apparently true rough colonies.

The Sonnei II Factor in Shigella dysenteriae - In Figure 5 is graphically presented the relative strength of agglutinins present in phase-specific S. sonnei II serum for the homologous organisms and for S. dysenteriae phase II. When S. sonnei II antigen was employed for the absorption of Shigella dysenteriae, phase II, serum, no agglutinins remained for S. dysenteriae phase II. On the other hand, when S. dysenteriae, phase II was used for the absorption of S. sonnei phase II, serum, there remained some, though weakly reactive, agglutinins for S. sonnei, phase II.

Figure 5

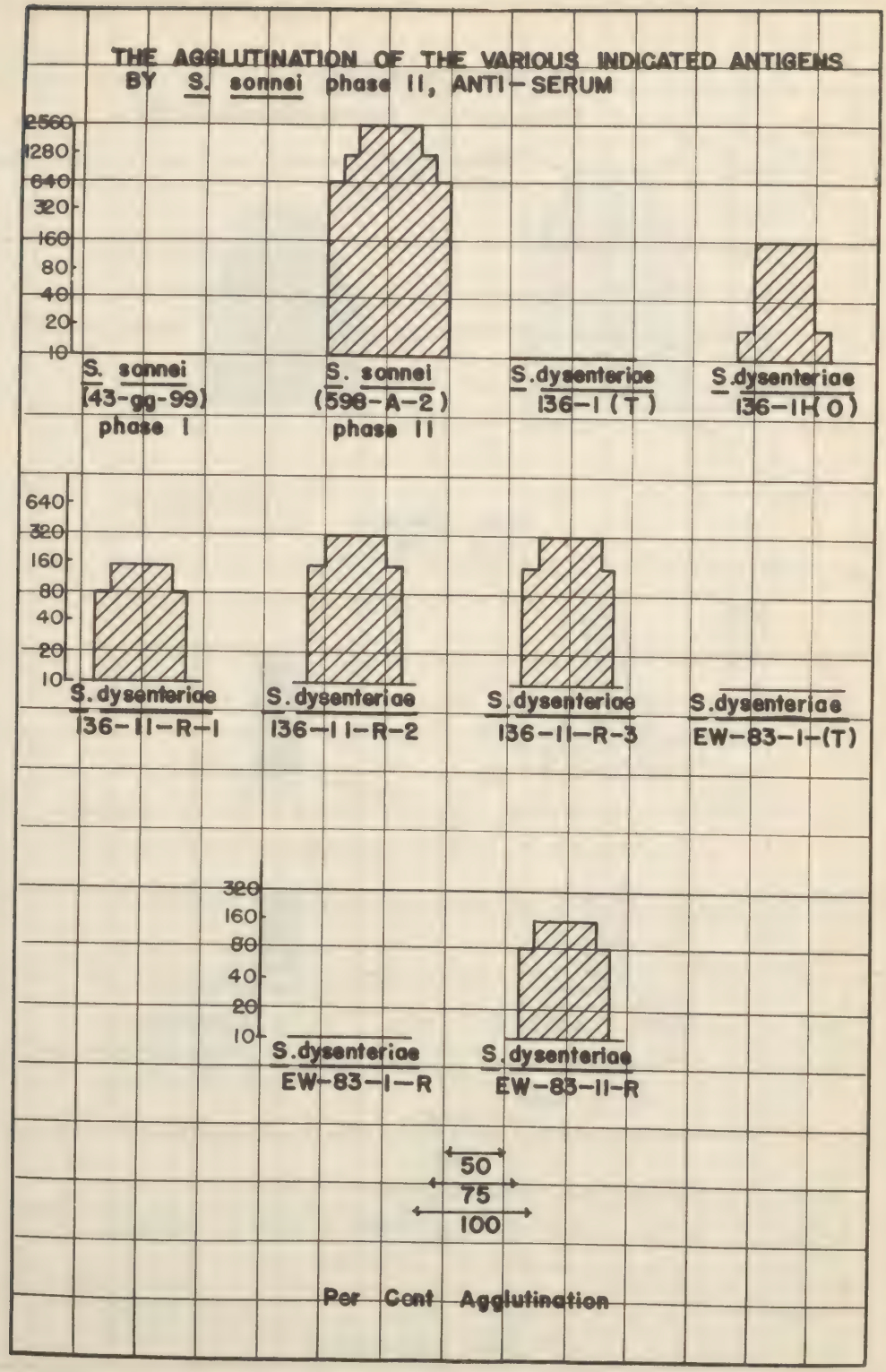
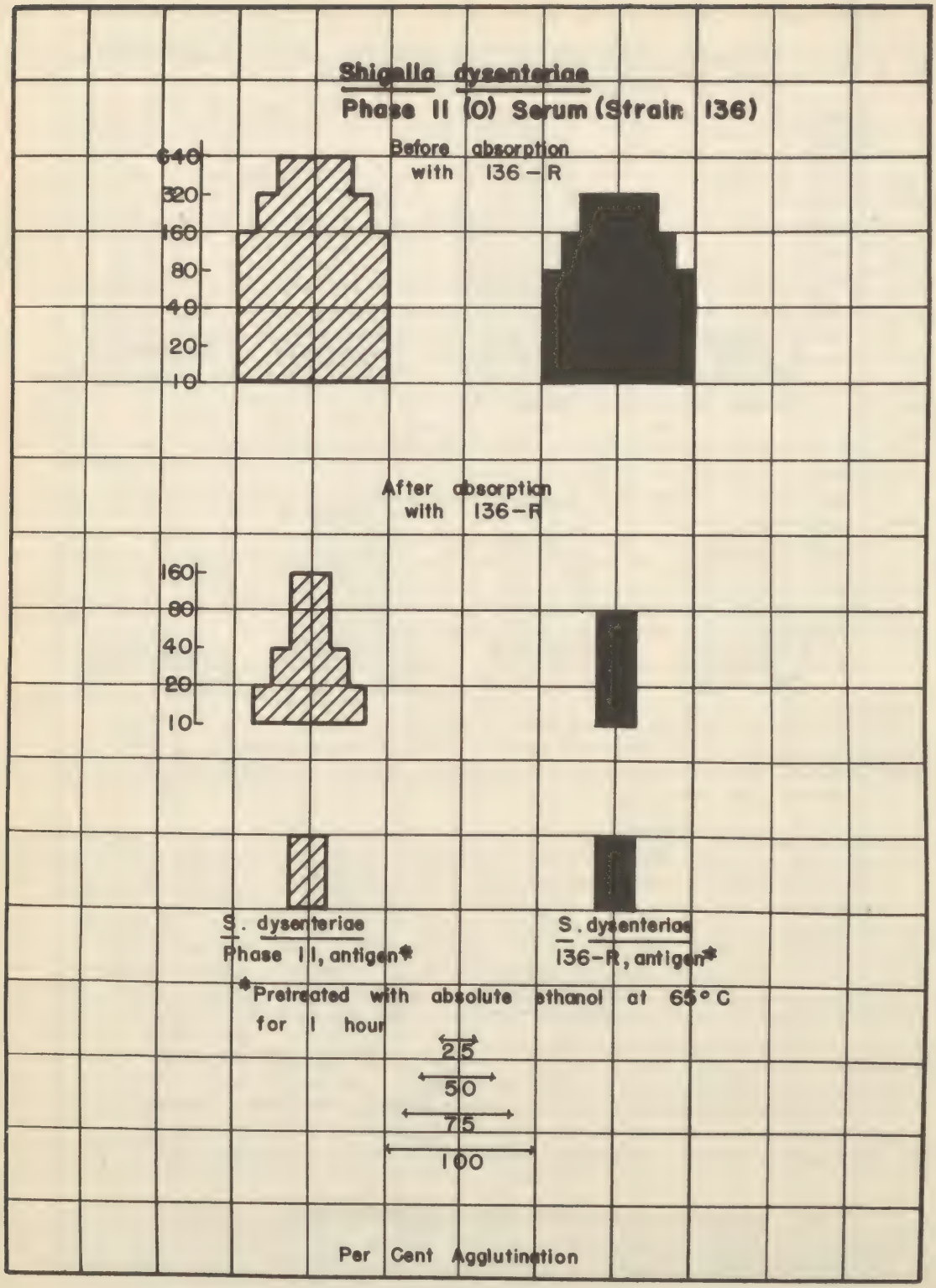

THE AGGLUTINATION OF THE VARIOUS INDICATED ANTIGENS BY S. sonnei phase II, ANTI-SERUM
2560
1280
640
320
160
80
40
20
10
S. sonnei (43-gg-99) phase I
S. sonnei (598-A-2) phase II
S. dysenteriae 136-I (T)
S. dysenteriae 136-II (O)
640
320
160
80
40
20
10
S. dysenteriae 136-II-R-1
S. dysenteriae 136-II-R-2
S. dysenteriae 136-II-R-3
S. dysenteriae EW-83-I-(T)
320
160
80
40
20
10
S. dysenteriae EW-83-I-R
S. dysenteriae EW-83-II-R
50
75
100
Per Cent Agglutination

Figure 6


Shigella dysenteriae
Phase II (O) Serum (Strain 136)
Before absorption with 136 – R
640
320
160
80
40
20
10
After absorption with 136–R
160
80
40
20
10
S. dysenteriae Phase II, antigen*
S. dysenteriae 136–R, antigen*
*Pretreated with absolute ethanol at 65° C for 1 hour
25
50
75
100
Per Cent Agglutination

Mouse Virulence and Phase Variation - Phases I and II of strains 136 and 167 were tested for relative virulence in the following manner. Broth consisting of 2% proteose peptone #3 (Difco), 5% serum, 5% yeast extract and 0.1% mucin was inoculated with the already mentioned Shiga bacilli and incubated for 6 hours, only, in an effort to minimize the exotoxin formed. The dilution of the culture in each case was made in a 5% mucin suspension. The results are shown below.

(1) Virulence test of S. dysenteriae, phase I (T), strain 136.

Dilution of Culture	10^{-1}ml.	10^{-2}ml.	10^{-3}ml	10^{-4}ml.
number alive / number injected	0/6	1/6	2/6	5/6

(2) Virulence test of S. dysenteriae, phase II (O), strain 136.

Dilution of Culture	10^{-1}ml.	10^{-2}ml.	10^{-3}ml.	10^{-4}ml.
number alive / number injected	2/6	6/6	6/6	6/6

(3) Test of filtrate for Exotoxin (dose: 0.5 ml.)

Strain	136-I-(T)	136-II-(O)
number alive / number injected	6/6	6/6

(4) Virulence test of Sh. dysenteriae, phase I, strain 167.

Dilution of Culture	10^{-1}ml.	10^{-2}ml.	10^{-3}ml.	10^{-4}ml.
number alive / number injected	0/6	0/6	0/6	6/6

(5) Virulence test of Sh. dysenteriae, phase II, strain 167.

Dilution of Culture	10^{-1}ml.	10^{-2}ml.	10^{-3}ml.	10^{-4}ml.
number alive / number injected	3/6	6/6	6/6	6/6

(6) Test of filtrate for Exotoxin (dose: 0.5 ml.)

Strain	167-I	167-II
number alive / number injected	6/6	6/6

There was apparently no appreciable amount of exotoxin formed. The LD_{50} for 136-I (T) was computed to be $10^{-3.25}$ or 1:1780, whereas that for 136-II (O) was $10^{-1.25}$ or 1:17.8. Comparing phase virulence of strain 136 phase I was 100 times more virulent for mice than was phase II. Phase I of strain 167 in like manner was found to be 320 times as virulent for mice as phase II of that strain.

Repeat determinations at a later date, using 10 mice per test and taking readings at 48 hours for 136, and 72 hours for EW-83 again show greater virulence of phase I endotoxin (Table 5).

Table V. Virulence of S. dysenteriae endotoxin

Strain	LD_{50} Phase I	LD_{50} Phase II
136	$10^{-3.896}$	$10^{-1.714}$
		$10^{-1.167}$
		10^{-1}
		$> 10^{-1}$
EW-83	$\frac{10^{-2.444}}{10^{-3.0}}$	$> 10^{-1.167}$

Tuberculosis: An Evaluation of Available Methods for Laboratory Diagnosis - To facilitate the diagnosis of tuberculosis an effort has been made during a 20 month period to evolve a technique which would furnish an irrefutable diagnosis and also hasten the time of a preliminary tentative diagnosis in order to permit the evacuation of such patients. Since most of the specimens presumably were submitted on a "rule-out" basis, methods for detecting a minimum number of organisms were desired.

For purposes of this study and for the final reporting of an organism as Mycobacterium tuberculosis only those organisms capable of producing histologic evidence of tuberculosis in guinea pigs (coupled with the demonstration of acid-fast bacilli in such lesions) are considered as "tubercle bacilli". No attempt was made to differentiate human from bovine types of M. tuberculosis.

The use of 4% sulfuric acid digestion under carefully controlled conditions yielded a concentrate with little extraneous material remaining. In an effort to obtain an even distribution of acid-fast bacilli violent agitation was introduced prior to a division for the several inoculations.

To permit a tentative diagnosis in a short time, the Tween 80-albumin medium of Dubos (12) was incorporated into the procedure. This medium had been utilized by

Foley (13) who considered that it could be successfully employed for routine diagnostic purposes. Other authors question the value of this medium as a diagnostic aid.

Corper's glycerol-egg yolk medium (14) was utilized as a second culture material. It must be emphasized that in the presentation to follow the value of Corper's medium, per se, is not under consideration since a constant supply of fresh eggs was not always available. This difficulty also eliminated Lowenstein-Jensen medium from consideration and actually was the original stimulus to attempt to utilize a wholly synthetic formula.

Two guinea pigs were inoculated from each concentrate. In the event that cultures were positive and the original pigs were negative the cultures were then injected into additional pigs to determine pathogenicity. All animals were tuberculin tested (old tuberculin) and the time of sacrifice controlled thereby. Animals showing a ten mm skin reaction following the injection of 0.1 ml of old tuberculin invariably showed histologic evidence of tuberculosis. It was thus frequently possible to shorten the period of observation to four or six weeks. At the end of eight weeks all animals were sacrificed for histologic examination.

The amount of concentrate from each specimen varied in amount. Effort was made to divide the material into four equal parts. This was only roughly quantitative. Dubos medium was never inoculated with more than 0.5 ml of concentrate per 5 ml. of media.

After elimination of several specimens in which the original inoculated animals had been lost due to non-specific causes the following data on 146 cases is available.

Sputa - 83

	Corper's (+)	Corper's (-)	Total
Dubos (+)	34	23	57
Dubos (-)	6	20	26
Guinea pig (+)	40	43	83

Non-sputa - 63

Dubos (+)	26	12	38
Dubos (-)	11	14	25
Guinea pig (+)	37	26	63

It is now possible to attempt to evaluate the relative efficiency of the various methods of forming component parts of the overall scheme under the conditions prevailing in this laboratory. Comparison will be made in chances of recovery by any single method versus the entire scheme and between various component parts of this scheme.

In reviewing the results obtained from sputa, it was found that the guinea pigs originally inoculated with the concentrate from each of 3 specimens failed to develop evidence of tuberculosis, yet acid-fast organisms were recovered by culture. Inoculation of additional guinea pigs with these cultures resulted in development of definite tuberculosis. Therefore, original inoculation of two guinea pigs per specimen resulted in recovery in 96.5% (80/83). Dubos medium alone afforded a 69.5% (57/83) recovery, and Corper's medium alone afforded a 48% (40/83) chance of recovery. Combined culture on Dubos medium and Corper's medium resulted in a 72.5% (60/83) chance of recovery. Assuming the recovery index in guinea pigs to be 1.00, the recovery index for Dubos medium alone is 0.71 (57/80), for Corper's alone is 0.50 (40/80), and for the combination of the two media the index is 0.79 (63/80).

Similarly, it was found that for non-sputum specimens the guinea pigs originally inoculated with the concentrate from each of 11 specimens failed to develop evidence of tuberculosis, yet acid-fast organisms were recovered by culture. Inoculation of additional guinea pigs with these cultures resulted in development of definite tuberculosis. Therefore, original inoculation of two guinea pigs per specimen resulted in recovery in 82.0% (52/63), Dubos medium alone afforded a 60.4% (38/63) recovery and Corper's medium alone afforded a 59.0% (37/63) chance of recovery. Combined culture on Dubos medium and Corper's

medium resulted in a 78.0% (49/63) chance of recovery. Assuming the recovery index in guinea pigs to be 1.00, the recovery index for Dubos medium alone is 0.73 (38/52), for Corper's alone is 0.71 (37/52), and for the combination of the two media the index is 0.94 (49/52).

It is desired to re-emphasize that the term "positive" as used refers to an organism which will produce histologic evidence of tuberculosis in the guinea pig with demonstrable organisms in the tissue section. The adoption of such criteria undoubtedly penalized the cultural methods since it assumed 100% development of disease in animals when inoculated with pathogenic organisms. Every effort was made to approach this degree of accuracy but the exact factor that did obtain remains problematical.

It is readily apparent that no single procedure used, nor partial combination of procedures is as effective as the complete routine. How much the incorporation of four procedures, perhaps all of equal value, would increase the likelihood of recovery over a single procedure cannot be assessed on the basis of information presently available. Certainly some improvement should be expected on this basis alone.

Interpretation is complicated further by the question of division of small amounts of inocula, particularly when relatively few organisms are present. The finding that the use of dual media produced almost the same percentage of recovery from non-sputum samples as did the use of dual pigs suggests that this factor should be considered. However, the use of guinea pigs alone for sputum samples appeared to enhance the chances of recovery when compared to the use of dual media.

In actual practice parts of the above paragraphs are of purely academic interest because the comparisons are based on the assumption that each attempt at culture or guinea pig inoculation will be carried through to a satisfactory conclusion. It does not take into consideration the possibility of non-specific deaths in guinea pigs or their loss in epizootics. It does not take into consideration the possibility of slightly sub-standard batches of media, variations in incubator temperature, breakage, or contamination. In evolving a satisfactory routine procedure all of these eventualities must be considered. The use of multiple methods thus permits a certain amount of salvage in the event one method is a complete failure due to accidental termination.

Most of the recent papers on the laboratory diagnosis of tuberculosis deals with the relative merits of one <u>in-vitro</u> medium versus another <u>in-vitro</u> medium, rather than their relative merits compared to an <u>in vivo</u> test. Foley (13) notes that of 57 specimens positive by culture and guinea pig only 50 (87.7%) were positive by culture alone, while 54 (94.7%) were positive by guinea pig only. Most standard textbooks do not equivocate as to the value of animal inoculation in comparison to cultural methods but newer media are not necessarily considered therein. Even the most enthusiastic reports merely indicate that "cultivation techniques are <u>as efficient</u> for diagnosis as is animal inoculation (with the exception of urine and gastric washings)." This same author indicates that "final typing" must be made in laboratory animals.

It is difficult to accept the concept advanced by some that differentiation of pathogenic from non-pathogenic acid-fast organisms can be made on the basis of morphologic appearance of the colony on culture media. Presumably the advocates of this method would so select strains for animal inoculation. Such a practice seems directly contrary to methods in vogue in other fields of bacteriology where diagnostic emphasis is placed on biological characteristics rather than on morphology.

The source of specimens and/or the selections of cases for study can definitely influence the relative merits of any method is more likely. Thus to contrast data derived from a study of patients in a sanitorium for tuberculosis and the data derived from a study of other type patients would certainly be a dubious method of ascertaining the value of any particular laboratory procedure.

There seems to be little argument but that a combination of cultural and animal inoculation procedures will result in a higher percentage of recoveries than any partial procedure included therein. Neither method is perfect and it remains for a carefully controlled study with known numbers of organisms to demonstrate the single method which will give the highest number of recoveries. The data reported herein indicates that in a laboratory diagnostic routine the chance of recovery from sputum samples was greater by dual animal inoculation than by dual culture while from non-sputum samples the likelihood was approximately equal. The question of the expense of routine examination by the combination of methods described must be considered in the light of the value of unequivocal rapid positive reports in the early recognition and treatment of patients.

Finally, certain parts of this diagnostic routine are intended to furnish an early tentative diagnosis. For this purpose the medium of Dubos' is of definite value. An effort was made to further shorten the time required for a "positive" diagnosis by inoculating the organisms obtained on culture into guinea pigs. This proved to be of no definite value over a series of several months and was eliminated. Perhaps with other animals or with adjuvants this possibility should be re-investigated.

An Improved Method of Antiserum Production - The method of Holstein (15) employing a 10% dextrose solution, administered intravenously, in stimulating antibody response for the production of antisera has been undertaken experimentally in this laboratory. Both rabbits and guinea pigs were used and comparative results are shown in Table VI.

Table VI. Results with One Initial Injection of 10% Dextrose Solution

Rabbit											Guinea Pig										
	Sh. paradysenteriae W-a																				
Animal	Dil. 1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	Animal	Dil. 1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
#2B (IV)	4	4	4	4	4	4	4	4	4	-	#1 (IP)	3	4	4	4	4	4	3	2	1	-
											#2 (IC)	4	4	4	4	4	4	4	3	2	-
	Sh. paradysenteriae W-b																				
#3-b (IV)	4	4	4	4	4	4	4	4	4	-	#3* (IP)										
#4-B (IV)	4	4	4	4	4	4	4	4	4	-	#4 (IC)	4	4	4	4	4	4	4	2	3	-
	Sh. sonnei II																				
#5-B (IV)	4	4	4	4	4	3	3	2	1	-	#5 (IP)	4	4	4	4	4	3	2	≠	-	-
#6-B (IV)	4	4	4	4	4	3	2	2	1	-	#6 (IC)	4	4	4	4	3	2	-	-	-	-

Rabbits were administered 10.0 cc of a 10% dextrose solution, intravenously, two hours prior to intramuscular injection of Shigella paradysenteriae W-a, W-b and sonnei II antigens. All animals were trial bled 12 days following injection of the initial 0.5 cc. dose of antigen and were bled-out on the 13th day.

Guinea pigs were given 2.5 cc. of a 10% dextrose solution, both intracardiac and intraperitoneally, two hours before the subcutaneous injection of Shigella paradysenteriae W-a, W-b and sonnei II antigens. They were trial bled 12 days following injection of the initial 0.5 cc dose of antigen and bled-out on the 15th day.

The response of Shigella sonnei II in both rabbits and guinea pigs was not favorably comparable to that obtained with Sh. paradysenteriae W-a and W-b. We, therefore, followed the protocol reported by Holstein (op. cit.) and administered the 10% dextrose solution two hours prior to the injection of each dose of antigen. These animals were also bled-out 15 days following the initial antigen and gave titers comparable to those obtained with strain W-a and W-b (Table VII).

Employing the above method it is now possible to obtain antisera with a titer of 1:5120 nine days following administration of the initial antigen. Until this experimentation was accomplished we considered an antisera showing a 1:1280 titer at the end of 29 days acceptable.

The efficacy of this method in producing high titer antisera with single strains is evident. Further investigation ensues with regard to the application of this method to the production of group antisera.

Table VII. Results when 10% Dextrose Solution was Administered Prior to Each Injection of Antigen

Rabbit											Guinea pig										
	Sh. sonnei II																				
Animal	Dil. 1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	Animal	Dil. 1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
#1-AC (IV)	4	4	4	4	4	4	4	4	3	-	#1 (IP)	4	4	4	4	4	3	2	1	/	-
#2-AC (IV)	3	3	3	3	3	4	4	4	4	-	#2 (IC)	4	4	3	3	3	2	1	/	-	-

* Died
IV Intravenous
IC Intracardiac
IP Intraperitoneal

PATHOLOGY SECTION

Under the provisions of AR 40-410 and GHQ, FEC Circular 69, this section continued to function as a histopathology center in 1949. Histopathologic service was furnished all hospitals in Japan throughout the year. With the closure of the 382nd Station Hospital in Korea in the spring and the 22nd General Hospital in Guam in the fall, histopathologic service for these hospitals was terminated. With the deactivation of the 3rd Medical General Laboratory in June this section assumed the responsibility for histopathologic service for the 34th General Hospital in Okinawa and as a reviewing and consultation agency for the Pathology Section of the laboratories in the United States Army Philippine Scout Hospital at Manila, P.I. and the 24th Medical Group at Clark Field, P.I. Histopathologic service connected with such tissue examinations as are required for various laboratory examinations in the Medical Zoology, Bacteriology and Virus and Rickettsial Sections of this laboratory has continued.

Routine

Pathologic material processed by this section can be considered under the following major classifications:

Human Autopsies	430
Human Surgical Pathologic Examinations	3198
Miscellaneous Examinations	1882
Total	5510

This represents an increase of 9.4% in autopsies over 1948, an increase of 18.0% of human surgical pathologic examinations over 1948, a decrease of 15.7% for miscellaneous examinations from 1948 and an overall increase of 3.8% in total examinations over 1948.

Human Autopsies - The policy of evacuating to the Zone of the Interior all transportable cases with serious illnesses as in the past continued to reflect itself in a high proportion of violent or unnatural deaths and deaths from coronary arteriosclerosis with or without thrombosis, and acute infections in our autopsy series and a low incidence of chronic disease. Trauma, by far, continued to be the largest single factor.

A tabulation of autospy material follows:

Trauma	116
Gunshot Wounds	44
Stillbirths and prematurity	44
Drowning	44
Coronary Arteriosclerosis with and without thrombosis	30
Malignant neoplasms	14
Encephalitis, etiology undetermined	14
Diphtheria toxoid poisoning	10
Acute pneumonitides	8
Burns	8
Congenital malformations	7
Ethyl alcohol poisoning	8
Stab wounds	6
Erythroblastosis fetalis	5
Meningitis, non-tuberculous	5
Methyl alcohol poisoning	4
Japanese B encephalitis	4
Peptic ulcer and complications	4
Acute anterior poliomyelitis	4

Cirrhosis of liver	4
Cerebral vascular accidents	3
Myocarditis	3
Barbiturate poisoning	3
Electrocution	3
Carbon monoxide poisoning	2
Aspiration of vomitus in infants	2
Measles	2
Diabetes mellitus	2
Pulmonary tuberculosis	2
Tuberculous meningitis	2
Diphtheria	2
Amebiasis	2
Acute panniculitis	2
Acute bacterial endocarditis	2
Anesthetic accidents	2
Phosphorus poisoning	1
Cyanide poisoning	1
Hanging	1
Motion sickness	1
Blood transfusion reaction	1
Pulmonary embolism	1
Septicemia, gross only	1
Pertussis	1
Amyotonia congenita	1
Lupus erythematosus	1
Volvulus	1
Pyelonephritis	1
Aplastic anemia	1
Appendicitis with rupture	1
Periarteritis nodosa	1
Acute hepatitis	1
Mesenteric thrombosis	1
Bronchial asthma	1
Dissecting aneurysm of aorta	1
Undetermined or incompletely studied	4
Total	430

Of 430 autopsies, 231 deaths were due to unnatural causes such as violence or poisoning and 199 deaths to natural causes. Of the 430 autopsies, 175 were done in the Tokyo-Yokohama area by members of this section; in the remaining 255 cases this section was responsible for the histopathology, the gross autopsy having been performed elsewhere.

The high incidence of violent or unnatural deaths, all of which must be handled as medico-legal cases, brings our section into close contact with various line-of-duty investigating officers and even in closer liaison with agents of various Criminal Investigation Divisions of various Provost Marshals. The pathologic management of these cases which causes a careful search for antecedent disease to be made in addition to evaluating the acute traumatic lesions, has enriched the educational experience of our officers in one of the most difficult fields in pathologic anatomy and military medicine, experience which can not be duplicated or offered in most civilian hospitals.

The resources of the X-Ray service of the Tokyo General Hospital, the Chemistry Section and the Virus and Rickettsial Section have been used fully to investigate deaths and to reduce the number of unexplained deaths to an absolute minimum.

Experience with Japanese B encephalitis cases in 1948 and 1949 at present supports the view that in the absence of an epidemic it is practically impossible to differentiate Japanese B encephalitis from bulbar poliomyelitis on histopathologic examination alone, and that success or failure depends almost entirely on proper and timely collection of specimens for isolation of the virus by virologic methods.

Careful studies of material received in ten cases of fatal diphtheria toxoid poisoning referred to in Annual Report for 1948 were most unrevealing.

Human Surgical Pathologic Examinations - Malignant neoplasms were encountered in 77 specimens of the total of 3198 specimens examined during the year. The types and locations of malignancies are shown in the table below:

Basal cell carcinoma of skin	28
Squamous cell carcinoma of skin	3
Basal-squamous carcinoma of skin	1
Squamous cell carcinoma of lip	2
Squamous cell carcinoma of cervix	7
Adenocarcinoma of breast	3
Adenocarcinoma of stomach	1
Carcinoma, undifferentiated, of stomach	1
Adenocarcinoma of uterus	1
Adenocarcinoma of colon	1
Adenocarcinoma of thyroid	1
Papillary cystadenocarcinoma of thyroid	1
Papillary cystadenocarcinoma of ovary	2
Adenocarcinoma of rectum	1
Embryonal carcinoma, testis	1
Seminoma of testis	1
Carcinoma, hypernephroid type, in liver	1
Carcinoma, undifferentiated, secondary	5
Adenocarcinoma in lymph node, secondary	1
Lymphosarcoma, lymph nodes	2
Hodgkin's disease, lymph nodes	4
Melanoma, malignant, skin	4
Melanoma, malignant, secondary, in lymph node	1
Myxosarcoma of thigh	1
Fibrosarcoma of leg	2
Fibrosarcoma of eyelid	1

A large number of specimens were appendices, 1364 out of a total of 3198. A high percentage, 88%, of these showed evidence of disease warranting operation. Endometrial biopsies and incomplete abortions accounted for 454 examinations. A variety of dermatoses and benign cutaneous neoplasms accounted for 430 examinations. The amount of time required to work up cutaneous material has been much greater than the number of examinations would indicate due to the difficulty of dermatopathology itself, particularly when the patients are seldom, if ever seen by the pathologist before removal of the lesion.

Miscellaneous Examinations - The tabulation below of miscellaneous examinations performed illustrates the scope of these examinations:

Guinea pigs for tuberculosis	1798
Canine brains for rabies	27
Mouse brain for rabies	3
Mice for schistosomiasis	22
Rabbit for bilharziasis	8
Human brains for encephalitis	12
Equine brains for encephalitis	5
Monkey brain for rabies	1
Rabbit, coccidiosis	1
Rabbit, variola	1
Chick embryo, variola	1
Conjunctival smear, trachoma	1
Mouse, carcinoma	1
Bone marrow, human	1
Total	1882

The decrease in miscellaneous examinations in 1949 is almost entirely due to the change in policy in the Bacteriology Section by which guinea pigs failing to develop a positive tuberculin test after inoculation, and failing at autopsy to show gross evidence of disease are no longer submitted for histologic examination for tuberculosis unless the material from which the inoculum was prepared was positive either by smear or cultural methods. Out of 1798 guinea pigs examined histopathologically in 1949, 168 were positive for tuberculosis.

Out of 27 dog brains examined for evidence of rabies in 1949 evidence of rabies was demonstrated in 5 instances, a sharp decrease in the number of examinations from 1948 when 60 dog brains were examined histologically.

Liaison with Other Agencies - During 1949 material and all available data have been sent to the Armed Forces Institute of Pathology in 427 autopsies and 1143 surgical specimens. The large number of surgical pathology specimens reflects our policy of sending to the Armed Forces Institute of Pathology all specimens of any professional interest or of any possible future administrative importance. Glass containers having been the cause of breakage previously, shipment of wet tissue to the Armed Forces Institute of Pathology are now made in metal containers.

Early in 1949 this section assumed responsibility for performing autopsies for the entire Tokyo-Yokohama area and the quality of work performed for the 155th, 128th, 5th and 376th Station Hospitals has been improved considerably over previous years.

Relations with the Tokyo General Hospital and 361st Station Hospital here in Tokyo have been excellent on the whole.

During the year the Chief of Section has served as Pathologist and Recorder on the Tumor Board of Tokyo General Hospital.

Training Activities - There has been sustained improvement in quality and quantity during the year. Beginning in September the bimonthly conferences with the Surgical Service of Tokyo General Hospital were increased to weekly conferences. Only in rare instances has it been necessary to run a dry conference using material from civilian stateside hospitals The use of the Bausch and Lamb microslide projector has been quite satisfactory.

Several clinical pathologic conferences have been held with the Medical Service, Tokyo General Hospital, using autopsied cases from their service.

A number of clinical pathologic conferences have been arranged for presentation before all medical officers in the Tokyo-Yokohama area with the Surgeon General's various civilian consultants in internal medicine. One such conference was held at the Eighth Army Medical Conference in Kyoto in November and appeared in the Bulletin of the Surgeon, Far East Command in January in 1950.

Special Projects - A combined study of surgically removed appendices by the Medical Zoology Section and Pathology Section was inaugurated in 1949 to determine the incidence of protozoal and helminthologic infections diagnosable by parasitologic and pathologic techniques.

During 1949, 26 appendices, or 4.1%, out of a total of 629 examined by parasitologic and pathologic techniques were found to be parasitized whereas in 1948, 19 appendices, or 1.8%, out of a total of 1028 examined by pathologic methods alone were found to be parasitized. The types of parasitizations encountered in 26 different appendices and the methods by which found in 1949 are tabulated on the following page:

Type of Parasitization	Found by both Pathologic & Parasitologic Techniques	Found only by Pathologic Techniques	Found only by Parasitologic Techniques	Total Found by Pathologic and Parasitologic Techniques
Ascaris lumbricoides	4	1	2	7
Hookworm	1	0	3	4
Trichuris trichiura	0	1	3	4
Enterobius vermicularis	1	2	1	4
Schistosoma japonicum	0	1	0	1
Endamoeba coli	0	0	3	3
Endolimax nana	0	0	3	3
Giardia lamblia	0	0	5	4
Total	6	5	20	31

It is of considerable interest that no cases of amebiasis of the appendix were encountered in 1948 and 1949. It appears that parasitologic methods are much more successful in finding protozoa in appendices than pathologic methods and that combined study by pathologic and parasitologic methods increase the number of helminthologic infections diagnosed by a significant number above those diagnosed by pathologic methods alone. As a result of our experience we are adapting the combined methods of study as a routine practice.

Recommendations for Changes Requiring Action by Department of the Army - To facilitate compliance with paragraph 12, AR 40-410, 20 August 1948, it is recommended that Standard Form 515 (Tissue Examination) be changed so that a space will be provided for recording the service number of personnel of the Armed Forces.

To improve the practice of obstetrics in the Armed Forces, to reduce the incidence of stillbirths and to reduce infant mortality, better correlation between clinical and pathologic data is needed. To facilitate the pathologic study of autopsies on stillbirths and infants dying during the neonatal period definite information concerning the maternal history, prenatal record and labor record are essential to evaluation of pathologic findings in stillbirths, and in neonatal deaths the same data plus the clinical record of the infant from birth to death are essential. It is therefore recommended that paragraph 8, AR 40-410, 20 August 1948 and paragraph 2b (2) TB MED 19, 15 January 1948 each be changed by adding the following:

"A clinical abstract in a stillbirth consists of a summary of the maternal history, prenatal history, labor record and such X-ray and laboratory examinations as may have been performed on the mother. A clinical abstract in a neonatal death consists of a summary of the maternal history, prenatal history, labor record and infant's history from birth to death and such x-ray and laboratory examinations as may have been performed on mother and child. The placenta and umbilical cord will be submitted for pathologic examination in every stillbirth and whenever possible in neonatal death."

It is requested that conflicting directions for the disposition of the original copy of autopsy protocols in paragraph 8, AR 40-410, 20 August 1948 and in paragraph 19b (1) AR 600-550, 23 June 1947 be resolved.

Change No. 1, paragraph 11, AR 40-410, 10 May 1949 provided that copies of reports from the Armed Forces Institute of Pathology will be made a part of the clinical record of the patient concerned. Reports received from the Armed Forces Institute of Pathology frequently concern a great number of patients. This requires a great amount of time to be spent by typists preparing extracts of reports from the Armed Forces Institute of Pathology for inclusion in the clinical records of the patients concerned. It also delays receipt of the report by the hospital patients concerned. This wastage and delay could be eliminated by the use of a standard form by the Armed Forces Institute of Pathology for each patient. The original report from the Armed Forces Institute of Pathology could then be forwarded by the contributing laboratory for inclusion in the clinical record and the copy retained by the contributing laboratory.

For intelligent evaluation of pathologic data obtained on autopsies on individuals dying outside hospitals, medical officers performing and interpreting autopsies need information which can only be furnished by summary court officers, line of duty investigating officers and Criminal Investigation Division agents. No administrative machinery exists in Army Regulations to effect liaison between these individuals and the medical officers responsible for autopsies. Such administrative machinery exists in the Far East Command and, if followed, works satisfactorily. It is therefore strongly recommended that the substance of paragraph 6b (2), Section II, GHQ, FEC, Circular 69, 1947 be incorporated within paragraph 19, AR 600-500, as delays in rendering autopsy reports are frequently due to the medical officer, of necessity, being required to evaluate his findings without the assistance of information already in the hands of investigators. Paragraph 6b (2) Section II, GHQ, FEC, Circular 69, 1947 reads as follows:

"Circumstances surrounding death. If the death occurred outside of a hospital, a statement concerning the circumstances surrounding death will be prepared by the investigating officer and submitted to the pathologist for inclusion in the protocol in lieu of the clinical abstract."

It is believed that this will improve the practice of medico-legal pathology in the Armed Forces, reduce delays in rendering autopsy reports to the minimum, better serve the ends of justice to the individual and the government than present practices in line-of-duty and criminal investigations, and assist in developing greater interest in the pathology of trauma, poisonings and obscure unattended deaths.

VIRUS AND RICKETTSIAL SECTION

This section provides facilities for confirmation of diagnosis of the more common viral and rickettsial diseases capable of practical laboratory examination. This includes primarily isolation, complement-fixation, neutralization, and agglutination inhibition procedures where applicable, against such agents as Japanese B, Western and Eastern, and St. Louis Encephalitis, Q fever, lymphocytic choriomeningitis, epidemic, murine and scrub typhus, and influenza.

In addition to such diagnostic services on a relatively routine basis, epidemiologic studies of any such diseases are conducted as permitted by space, material and personnel available.

The potential military importance of diseases of this type require continuing investigative studies which serve two purposes: (1) Collection of additional scientific data, and (2) training of personnel in this increasingly important field. The availability of material for such studies in this area is well recognized. The late summer appearance each year of Japanese B encephalitis results in its recognition as of primary importance.

Japanese B Encephalitis - A historical review, report of previous experience and investigations with particular reference to the epidemic of 1948 has been reported (1a). Technics used likewise are included in the same report. Additional information only will be presented here. It would be well to compare this with last year's data. Japanese B encephalitis in the Far East - 1949: Although sporadic cases are reported in Japan throughout the year under this diagnosis, there is strong reason to believe true cases occur during a relatively limited period in July, August or September.

The earliest recognized outbreak of the disease occurred in Okinawa with a total of 38 Okinawan and 3 American cases. The first case in natives and all 3 in Americans began in June. Following subsidence in the Okinawan population in August and September, appearance was awaited in Japan.

In July and August, the usual sporadic "cases" were reported. In the first week in September reported cases showed a definite increase in clinical cases with date of onset of 29 August. After the usual rapid wave yielding 10 clinical cases of the disease in Americans and approximately 1300 in Japanese, the minor epidemic as such was over by the end of September.

During the same last week in August through most of September an epidemic of 5548 cases affected the native population of Korea. Inasmuch as Japanese B encephalitis in native Koreans has not been recorded previously in American or Japanese literature, this subject is discussed more fully below.

Japanese B Encephalitis in Americans During 1949 - There were 13 clinical cases of Japanese B encephalitis among American personnel, 10 occurring in Japan and 3 in Okinawa (Table I). One of the cases in Okinawa and two in Japan had a fatal outcome. Death occurred on the second, fourth and sixth day of the disease. In three cases in which relatively complete recovery did not occur, evacuation to the Zone of the Interior prevents final evaluation of residual damage. The remaining 7 cases showed complete recovery and were returned to duty. In evaluating damage it must be realized that indoctrination in control methods is often strenuous, and when "brain fever" is contracted, certain mental trauma is experienced on purely psychic grounds. Evacuation to the ZI undoubtedly occurs when such psychic trauma is sufficient to prevent resumption of normal military duties in this theater. Definite mental retardation apparently occurred in one case who experienced a temperature of 106°F for a period of 3 days during the active phase of the illness. The patient also was the only one to exhibit any residual motor damage, evidenced by a right sided hemi-paresis.

The 3 cases in Okinawa occurred in June with onset on the 8th, 13th and 17th of the month. Of the 10 cases in Japan the onset of one was 19 August, the others beginning between the 5th and 30th of September. Of the total 13 cases, 10 had received

Table I. JBE Cases in Americans 1949

Case No.	Initials	Location	Immunization Series	Immunization Stim. Dose	Date Of Onset	Date Of Adm.	Disposition
1	J.S.	Okinawa	1949		13 Jun	14 Jun	Death* - 14 Jun
2	H.M.	Okinawa	1949		17 Jun	24 Jun	Duty
3	E.B.	Okinawa	1949		8 Jun	12 Jun	Z.I.
4	W.B.	Japan	1949		30 Sep	2 Oct	Death - 3 Oct.
5	J.B.	Japan	Records lost in typhoon		8 Sep	13 Sep	Duty
6	D.B.	Japan	1948	1949	14 Sep	19 Sep	Z.I.
7	H.G.	Japan	1948	1949	13 Sep	17 Sep	Duty
8	W.H.	Japan	1949		6 Sep	7 Sep	Z.I.
9	J.L.	Japan	1949		16 Sep	19 Sep	Duty
10	C.M.	Japan	1949		16 Sep	18 Sep	Death - 21 Sep
11	J.M.	Japan	None	None	30 Sep	3 Oct	Duty
12	B.M.	Japan	1949		19 Aug	24 Aug	Duty
13	E.R.	Japan	None		16 Sep	20 Sep	Duty

Serologic Results

Date of Collection with Complement Fixation and Neutralization Index

Case No.	1	2	3	4	5	6	7	8	9
1									
2	25 Jun	5 Jul	12 Jul	19 Jul					
	0	1:16	1:16						
	>160	500	1600	63,000					
3	14 Jun	28 Jun	5 Jul	12 Aug					
	0	1:8	1:8	1:4					
	8,000		100,000	100,000					
4	2 Oct	4 Oct							
	0	0							
	130	250							
5	13 Sep	23 Sep	7 Oct						
	0	1:32	1:32						
	> 20,000		8,000						
6	19 Sep	29 Sep	15 Oct	18 Oct	22 Oct	26 Oct			
	0	1:16	1:16	1:16	1:8				
	4,000	< 20,000	13,000	< 2,000	>13,000	>13,000			
7	19 Sep	29 Sep	6 Oct	10 Oct	14 Oct	18 Oct	22 Oct	26 Oct	
	1:8	1:16	1:32	1:32	1:32	1:32	1:64	1:32	
	8,000	< 20,000	8,000	< 8,000	< 10,000	< 2,000	6,300	>13,000	
8	9 Sep	10 Sep	10 Sep	17 Sep	24 Sep	5 Oct	20 Oct	4 Nov	
	0	0	0	1:8	1:16	1:32	1:64	1:32	
	800	5,000		3,200			320		
9	20 Sep	1 Oct	5 Oct	10 Oct	14 Oct	17 Oct	22 Oct	26 Oct	
	1:4	1:32	1:64	1:128	1:128	1:64	1:128	1:128	
	13,000	13,000	< 13,000	< 8,000	< 10,000	< 2,000	> 13,000	> 13,000	
10									
11	4 Oct	10 Oct	12 Oct	17 Oct	21 Oct	24 Oct	31 Oct		
	0	0	0	1:8	1:8	1:16	1:8		
	13,000	2,500	> 16,000	10,000	16,000	> 13,000	> 20,000		
12	29 Aug	14 Oct	19 Oct	24 Oct					
	1:8	1:8	1:8	1:8					
		13,000		>13,000					
13	21 Sep	30 Sep	30 Sep	10 Oct	10 Oct	14 Oct	18 Oct	22 Oct	26 Oct
	0	1:8	1:16	1:4	1:8	1:4	1:4	1:8	1:8
	8,000		20,000	4,000	8,000	1,600	< 2,000	8,000	13,000

* Confirmation by isolation of JBE virus in each of 3 fatal cases

the prescribed vaccine, 2 had not been vaccinated, and in the remaining case loss of the immunization records prevented definite proof one way or the other.

These 13 cases were all confirmed by laboratory examination. Virus was recovered from brain tissue in each of the three fatal cases. The non-fatal cases all showed significant complement fixation titer and/or neutralization index changes. Case #12 is considered among the proven ones although without meeting the criteria for complement-fixing titer rise required for laboratory diagnosis. This patient also had an onset somewhat earlier than the rest of the cases in Japan, but one possible factor may be related to his being stationed at Gifu, whereas other cases in Japan were from areas slightly to the north. Including this individual, cases originated from areas of Honshu as follows:

Tokyo-Yokohama Area	7
Gifu Prefecture	2
Gunma Prefecture	1

Considering the combination of clinical and laboratory evidence of a virus infection of the central nervous system, plus the persistance of appreciable complement fixing and neutralizing antibodies, the JBE virus as the etiologic agent is felt to be established in case #12. His clinical resume follows:

Case No. 12, B.M. This 29 year old negro male was admitted to the 28th General Hospital 24 August, complaining of a headache, backache, and high fever of 5 days duration. The headache was usually generalized with periods of localization to the frontal areas. Slight chills and dyspnea had also been noted. Physical examination showed moderate fever, tachypnea of a rapid, shallow type, and nuchal rigidity. Peripheral blood showed WBC 9,700, neutrophils 60%, lymphocytes 34%, monocytes 4%, eosinophils 2%. Spinal fluid showed normal dynamics, WBC 135, neutrophils 18%, lymphocytes 82%, sugar 45 mgm%, total protein 42 mg%, culture, gold curve, and Kahn negative. Slight aching and weakness of left tricepts subsided with rest and hot packs. Temperature fell by lysis to normal by the 7th Hospital day, headache and nuchal rigidity disappeared, and patient was returned to duty after 17 days hospitalization. Subsequent receipt of report from this laboratory of positive complement-fixation test for JBE 1:8, led to his readmission and transfer to 361st Station Hospital, the installation charged with care and study of all JBE cases. No residual evidence of CNS disease could be found on careful study other than slight pleocytosis gradually clearing, and persistent serologic evidence of JBE as shown (Table I).

A summary of another of the cases is reported. Case No. 10, C.M.: This 19 year old American Indian male developed headache 16 September which persisted. He played an entire game of football the next day following which he noted increasing headache and pain in his legs. That night weakness in the legs increased to paraplegia, and he was hospitalized. Physical examination showed nuchal rigidity, positive Kernig's sign, negative Babinski, and absent patellar, cremasteric, and Achilles reflexes. No disturbance of sensation or clouding of sensorium was found. Peripheral blood showed WBC 16,150 with neutrophils 76%, lymphocytes 20%, monocytes 3%, eosinophils 1%. Spinal fluid revealed normal pressure; WBC 290 with 76% lymphocytes; sugar 45.6 mgm% and globulin negative. Temperature rose from 102.4° to 104.6°F and the following day an ascending paralysis led to inability to void, labored respiration, slurred speech, and difficulty in swallowing. Despite tracheotomy and administration of oxygen death occurred early on the third hospital day. Stupor was present only terminally. Autopsy showed findings consistent with JBE, and virus isolate was identified as that agent.

These two cases were both diagnosed poliomyelitis and are presented to emphasize the fact accepted locally that final differentiation of poliomyelitis and JBE is a laboratory procedure. It is for this reason that paired sera are submitted on all virus diseases of the central nervous system to rule the latter in or out.

It is not the purpose of this communication to describe in detail clinical aspects of the American cases. Adequate descriptions may be found elsewhere (6).

Serologic findings have been given above. In two instances, complement-fixing antibody titers were found prior to the fourth day of illness. Of these sera 3 of 11 (27%) from individual cases were positive between the 4th and 5th days after onset. From the 6th through the 10th day, only one additional serum was positive, while 4 sera tested between the 11th and 15th day of the disease were positive. Of the 11 sera tested, 3 more became positive between the interval of 16 to 20 days after the onset of illness. The results of this small group would indicate that a positive complement-fixing antibody titer could be expected in 70% of cases by the 15th day of the illness.

In all cases a positive neutralization index was demonstrated in initial sera drawn 2 to 8 days subsequent to onset of symptoms. This is a striking contrast to the accepted immunologic pattern as pointed out by Hammon, W. McD. (7). It is interesting to note that the development of complement-fixing and neutralizing antibodies closely approximates the human immunologic pattern as shown in Western Equine encephalomyelitis (7). The composite serologic pattern of this group of individuals corresponds rather closely to that of a single case reported by Sabin et al (4). It appears (Case #4, W.B.) that early development of neutralizing antibody does not prevent a fatal outcome or the ready recovery of virus from the brain.

Japanese B Encephalitis in Okinawans - The 38 reported clinical cases in native Okinawans showed a scattered distribution of cases throughout the main island with two cases reported from Kume-jima, a smaller island about 60 miles west of Naha. The usual picture of predominance of children and young adults was noted. Of 33 cases in which the outcome was known, 12 deaths occurred giving a 36% mortality.

Japanese B Encephalitis in Japanese - Although cumulative reports are not yet complete, statistics from Japanese health agencies through Public Health and Welfare Section, SCAP establish total reported cases as 1300 with 466 deaths for a case fatality rate of 36%. The distribution of cases by prefecture show the usual low incidence of cases in the northern prefectures of Honshu and in Hokkaido. Fifty-seven per cent of the prefectures produced 71 per cent of the cases during a period of 6 weeks (from 27 August to 8 October). A comparison of the number of cases of Japanese B encephalitis for comparable periods show that there were 264 cases reported in 1947, 7208 in 1948, and 1301 during 1949 with 133, 2930 and 475 deaths respectively for the periods described above.

The more available figures on occurrence of cases in Japanese are from Tokyo. These included clinically "confirmed" cases, based on initial public health reports plus subsequent corrections. Based on date of onset the epidemic in this locality is felt to have begun on 28 or 30 August, the peak of 17 cases in one day falling on 12 September, and return to the sporadic reports of single cases being 4 October (Fig. 1).

Laboratory confirmation of the epidemic will be discussed in general below.

Japanese B Encephalitis in Koreans - During the last week in August and the first week in September 1949, the Korean Ministry of Health received reports from small villages along the 38th parallel of an outbreak of a central nervous system disease, resembling meningococcic meningitis. Based on negative spinal fluid cultures, the differential of the spinal fluid counts, together with the clinical picture, the chief epidemiologist suggested that the outbreak was due to "summer encephalitis".

From its origin along the 38th parallel, the outbreak spread rapidly to Seoul where the epidemic was most severe. During the second and the third week of September, this city reported 1283 cases, and detailed investigation of 567 case reports showed the distribution to be consistent with the population density. During the latter part of September, the disease spread rapidly from the Seoul area, producing sporadic cases in all areas except on the island of Cheju-Do (Fig. 2). By 16 October the disease had abated. During the 51 days the disease had been recognized, a total of 5548 cases of encephalitis with a case fatality rate of 44 per cent was reported.

Clinical Findings - The following data were compiled by the chief epidemiologist, Ministry of Health, in which over 600 hospital charts were reviewed by him. Those that did not appear consistent with the disease were discarded. The remaining 567 cases were taken as a sampling survey and analyzed.

Figure 1

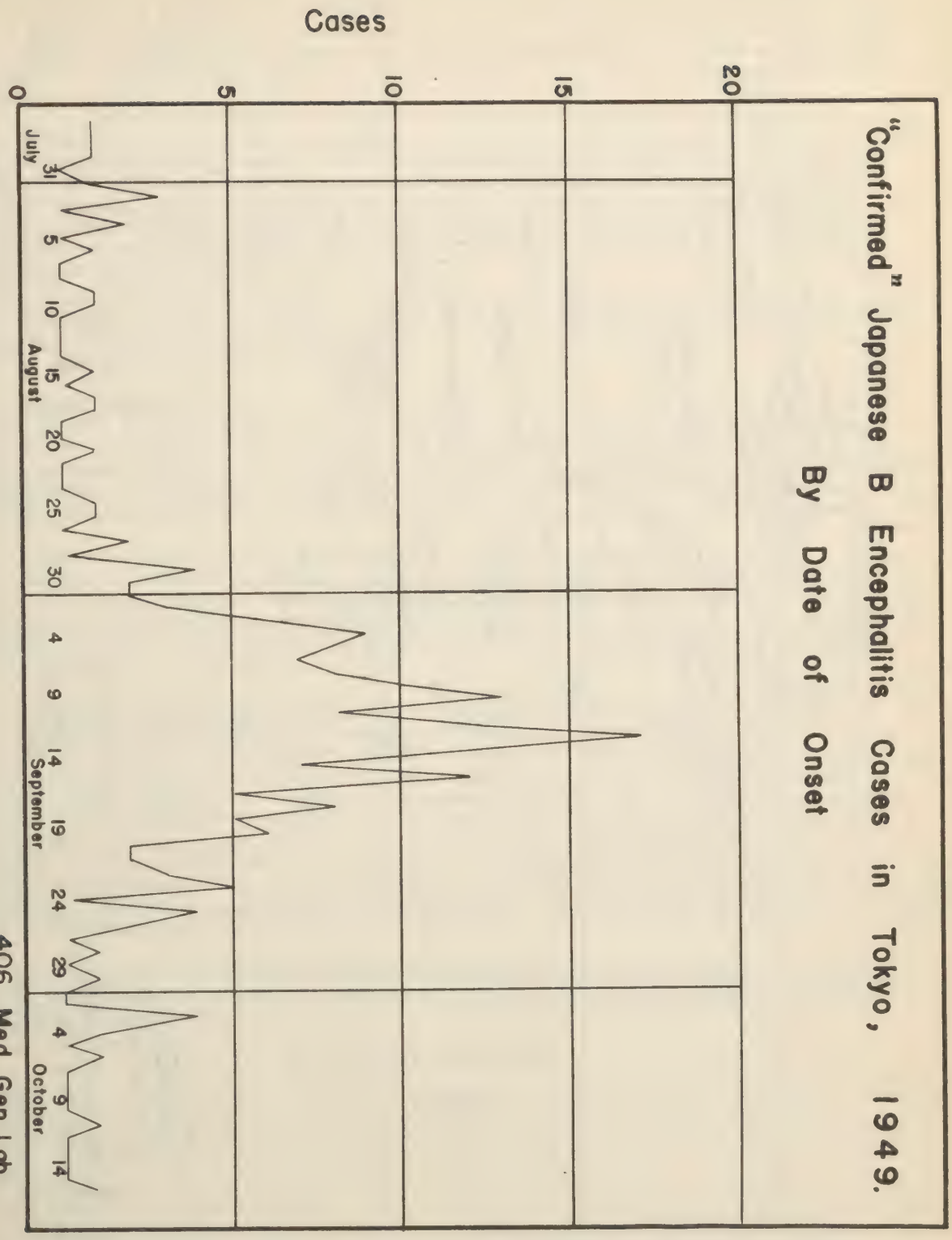

"Confirmed" Japanese B Encephalitis Cases in Tokyo, 1949.
By Date of Onset
Cases
0
5
10
15
20
July
31
August
5
10
15
20
25
30
September
4
9
14
19
24
29
October
4
9
14
406 Med. Gen. Lab.

Figure 2


KOREA
OUTLINE MAP
• PROVINCIAL CAPITAL
100 Miles
140 Kilometer
CHUNCHON
SEOUL
KANGWON - DO
KYONGGI - DO
CHUNGCHONG PUKTO
KYONGSANG-PUKTO
TAEGU
CHONGJU
TAEJON
CHUNGCHONG-NAMDO
CHONJU
CHOLLA-PUKTO
KYONGSANG-NAMDO
PUSAN
KWANGJU
CHOLLA NAMDO
CHEJU - DO
CHEJU
YELLOW SEA
KOREA STRAIT
JAPAN

Age and Sex - The age group (Japanese reckoning) 3 to 13 years constituted 63 per cent of the cases with 4 cases in one year olds, 16 cases in patients 60 years and above. Of the 567 cases, 57 per cent were males. This is in keeping with the apparent greater susceptibility of males noted in human epidemics in Japan. Suffice it to say the clinical aspects of all case reports correspond to those in which adequate descriptions have already been cited. It may be noted, however, that in comparison to the Japanese cases, the illness seemed more severe and stormy with a clouded sensorium appearing in some 69 per cent of the cases for varying periods of time.

Another item of note is the fact that at the time of discharge, a number of patients had residuals present. This was undoubtedly due to the length of hospitalization, in that the patient remained only through the most severe periods, which usually averaged 12 to 14 days. Fluid balance was not often maintained primarily due to economic factors. This fact, the nutritional background, and the limited nursing care available prohibit comparison of overall fatality rate with that found in American personnel and facilities.

Pathology - The usual characteristics of a virus encephalitis (perivascular cuffing, glial nodules, neuronal destruction and congestion) were present with prominence of widespread cortical involvement.

Virus Studies - In 11 fatal cases brought to autopsy, virus isolates were obtained from 7 individuals by Dr. Ryong Sook Lee in the laboratory of Seoul National University. From an original brain tissue preserved in glycerin and received in this laboratory on 9 September 1949, a neurotropic virus was obtained (V9-4067). Additional original material in glycerin from each of the other 6 cases was received by this laboratory on 4 November 1949, but isolations were not successfully accomplished.

A virus strain (V9-9172) was obtained from Korean passage material (mouse brain in glycerin). A second strain was isolated from Case #4 (V9-8421), which was forwarded to this laboratory, following inoculation of mice in Korea. These 3 isolates have tentatively been identified as Japanese B virus by complement fixation and neutralization tests. Cross protection studies with Japanese B encephalitis (Nakayama), SLE and WEE are in progress.

Serologic Virus Studies - Complement fixation data on 17 "typical convalescent patients" would suggest that the disease was caused by the virus of Japanese B encephalitis, although the incidence of 4 positive complement fixation tests (1:4 to 1:16) among 17 convalescents in contrast to one positive test among individuals without history of the illness leaves much to be desired. In view of the fact that the blood specimens were obtained between 15 and 23 days after onset of the illness, one would expect the majority of patients to have developed complement-fixing antibodies if this illness were due to Japanese B encephalitis. However, adverse conditions, namely poor refrigeration through power failures during the time the sera was held in storage undoubtedly reflected itself through a reduction in both frequency and degree of titres on the convalescent serum specimens.

Nineteen young presumably "normal" adults were bled toward the end of the epidemic and of these, one had a positive complement fixation test while 17 had significant neutralization indices.

Neutralization Indexes

	20	50-100	100-1000	10,000-20,000	20,000+
Convalescent			4	7	1
"Normal"	2	1	1	6	9

The neutralization indices reported on the above-mentioned convalescent blood sera are of the titre expected and compare very favorably with what was found on Okinawa and in Japan from 1945 to the present time. The titre of the convalescent serum from 2 to 6 weeks after onset is usually lower than the titre found in "normal" residents of the area who have antibodies. This tends to support the diagnosis of

Japanese B encephalitis for if it were an entirely unrelated infection, those people should have the same average titre as the "normal" persons.

Realizing the shortcomings of our initial survey, we returned to Seoul to collect additional data of epidemiological significance on the recent outbreak in this area. Individuals, immune because of previous experience to a live virus, may not develop complement-fixing antibodies as a result of new exposure to minimum quantities of the live virus, such as might occur through natural vector exposure. As was first noted by Hammon (8), the administration of a potent vaccine to individuals immune as a result of previous experience with a live virus, results in a prompt anamnestic response of non-vaccinated individuals (particularly children) to a single dose of Japanese B encephalitis vaccine.

Thirty-six orphan children between 4 and 15 years of age living in Seoul, Korea, received 1.0 ml subcutaneous dose of chick embryo vaccine, lot #302-E. In addition, 89 non-vaccinated Korean soldiers stationed as corpsmen at Capital Army Hospital, urban Seoul, participated in the program. Blood specimens (children 36/63* and Korean soldiers, 89/63) were obtained immediately prior to and 9 days following vaccination. All sera were separated in the usual manner and maintained in the frozen state until tested.

Paired specimens were simultaneously tested for complement-fixing and virus neutralizing antibodies. Prior to vaccination three soldiers showed complement-fixing titres of 1:4 while all of the orphans were negative. Nine days after receiving Japanese B encephalitis vaccine, 28 soldiers had complement fixation titres of 1:4 to 1:16 and 2 children had a post vaccination titre of 1:4 to 1:8. This response to vaccination was expected. The rapid and regular appearance of complement-fixing antibodies for the Japanese B virus, following vaccination of the soldiers who exhibited evidence of previous inapparent infection is in agreement with an observation made by Hammon, W. McD. (8) on a small group of Japanese adults in 1946.

On the other hand, the Korean children differed in their immunologic response in that only 2 of 20 children tested exhibited positive complement-fixing antibody titres. This is in agreement with an observation made by Sabin et al (9) in which none of the Japanese children showed a positive neutralization indices prior to vaccination, following which only one (1:4 (2/)) of 22 children developed a positive complement fixation titre 10 days subsequent to the second dose of vaccine.

Neutralization indices were done on a pre-vaccine sera on 73 soldiers. Fifty-seven of this group had indices ranging from 250 to 32,000 with the mean 20,000. All of the above-mentioned figures would suggest a widespread dissemination of the Japanese B encephalitis virus among the population during 1949.

During the same time in which the serologic survey was conducted, among the Korean population, animal sera was obtained from various species including chickens, horses, dogs, swine, cattle and sheep. Suffice it to say, a high incidence of neutralizing antibodies was observed among cattle, dogs, horses and swine. This would indicate a wide dissemination of Japanese B virus among Korean animals.

Positive complement fixation titres were observed in 5 out of 25 horses and in 6 out of 14 swine, adding considerable emphasis to the epidemiologic data, that not only was the virus disseminated during 1949 but its very presence could be indicated through the residual complement-fixing antibody titres among animals. Burns et al (10) have shown that equine complement-fixation titres are of a transitory nature, having a maximum duration of 12 to 16 weeks.

Routine

The routine diagnostic work performed may be summarized in Table II. This does not include the numerous similar procedures required in investigative work, in identification of isolates, or in preparation of biologics required for the above tests. Nor does it reflect the hours lost in handling the first and only specimen received in cases requiring paired sera.

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* The numerator denotes the number of first bleedings; the denominator signifies the individuals appearing for post-vaccination bleedings.

Table II. Rotuine Tests Performed

Virus Complement Fixation Test	4220
Virus Neutralization Test	2637
Virus Isolation Attempts	76
Typhus Complement Fixation Test	736
Influenza Agglutination-Inhibition Test	149

Complement Fixation Test for Japanese B Encephalitis - Paired blood specimens are received on all clinical cases of suspected virus infection of the central nervous system in American personnel. Due to the prevalence of JBE virus in this area, all such sera are tested for rise in complement fixing antibody titer to this virus. This includes, therefore, many cases of clinically typical poliomyelitis, an almost equally prevalent agent. Most of the material was submitted from Japanese cases during the epidemic season.

All complement fixation tests were carried out during the past year, using the technic described by Casals and Palacios (1) with all antigens being prepared in accordance with the method employed by Espana and Hammon (2).

The degree and frequency of titer rise in sera from suspect cases have not been as high as recorded for 1948. Apparently similar results by Japanese workers somehow led to a local rumor of the possibility of a new or intermediate strain of encephalitis virus. Following the demonstration in 1948 of the dependability of serologic confirmation, during the early stages of a threatened epidemic, concern over positive confirmation naturally lead to a desire to shorten the period required for laboratory diagnosis. The small and infrequent titer rises detectable in prematurely collected second specimens when compared with more deliberate collection in the later period of the 1948 epidemic explain the lower degree of titer rise and the readiness to question the suggested proof of the same etiologic agent. Such doubts were dispelled as more material became available. Our procedure has been altered this year only by standardization of incubation time following addition of an indicator system, and by reporting only the highest dilution showing a 3 or 4/ reaction. A case was considered serologically confirmed if a 4-fold rise in titre was exhibited. In no instance was any degree of partial fixation considered to have any significance if the degree of reaction was 2/ or less in a 1:4 dilution. All other procedures and materials remained as previously reported.

Sera from 1948 which have been re-tested according to the standardized system indicated above, show only a slight fall in complement-fixing titres from those serologic readings recorded last year. This was felt to be expected following a prolonged storage period. Comparison of results on material submitted to AMDR&GS shows good agreement. A total of 3640 sera were examined as 1820 paired specimens from human cases suspected of JBE virus etiology. Of these 1820 pairs, 259 showed a diagnostic rise in titer, 63 were anticomplementary, preventing comparison between members of the pair, and 1498 pairs were reported as negative. These are summarized in Table III.

Virus Isolations, JBE - Fatal human cases resulted in the submission of 42 specimens from autopsy suitable for attempts at virus isolation. These were received in a fresh state from the Tokyo area, or preserved in buffered or plain 50 per cent glycerin from other areas.

Virus isolations were accomplished either as a simple 10 per cent brain suspension inoculation of mice and guinea pigs and a combination of high speed centrifugation and/or antibiotic inhibition prepared of inoculums with inoculations being made intracerebrally and intraperitoneally.

Primary serological evidence of Japanese B encephalitis infection was attempted in each instance by the preparation of an antigen from the human brain material according to the method outlined by Sabin et al (4). In only 3 of 19 primary attempts were we able to determine evidence of infection serologically with the original autopsy material.

Table III. Routine Complement Fixation Tests for Japanese B Encephalitis

1949 Month	No. of Spec.	Clinical Specimens, Human Neg.	AC	Pos.	Jap. */	Am. **/
June 20 – July 20	388	328	16	44	41	3
July 20 – Aug. 20	84	80	1	3	3	0
Aug. 20 – Sept. 20	255	235	11	9	6	3
Sept. 20 – Oct. 22	169	113	2	54	12	3
Oct. 22 – Nov. 22	712	569	29	114	68	8
Nov. 22 – Dec. 30	212	173	4	35	29	0
Total	1820	1498	63	259	159	17

* Includes Okinawans
** Multiple specimens on American personnel

As a means of preliminary identification of isolates, antigens for use in complement fixation tests were prepared from infected mouse brain usually in the second or third successive mouse passages by emulsification of the brain to a 10 per cent suspension. After centrifugation, the supernatant was placed in lusteroid tubes and immersed in alcohol and solid CO_2 with alternate freezing and thawing until precipitation occurred. The supernatant fluid was used as an antigen and showed fixation properties to Japanese B hyperimmune sera in dilutions ranging from 1:32 to 1:128 (24). Controls consisting of Eastern and Western Equine encephalomyelitis and St. Louis hyperimmune serums were negative. Cross reactions with SLE were frequently observed in low dilutions (1:4).

In order to specifically identify the isolates recovered, cross resistance tests are being conducted with other neurotropic type viruses. Immunization studies for the identification of the isolates by protection tests for SLE, WEE and EEE hyperimmune serums are still incomplete.

Using these methods, isolates were obtained in 19 of the 42 attempts as summarized in Table IV.

JBE Vaccine Evaluation in Okayama - To ascertain the effectiveness of JBE vaccine in an endemic area a project was begun in 1946 in Okayama Prefecture. The results of the period 1946 through 1948 have been previously presented in some detail (1a). Immunization has been expanded in scope each year, previously immunized children receiving a single stimulating dose, and additional numbers receiving an initial series as shown in Table V.

Table IV. Human Neurotropic Isolates

No.	Name	Sex	Age	Source of Material	LD_{50}	Neut. Index JBE	C.F. Titre JBE	C.F. Titre SLE
				Americans				
V9-1721	Schoke, J.E.	M	20	Okinawa	5.5	320	1:32	1:2
V9-4902	Manford, Chief Eagle	M	Unk	Yokohama	7.2	400	1:64	1:2
V9-6314	Batt, W.	M	27	Tokyo	7.7	400	1:32	0
				Koreans				
V9-4067	Kwak, Kyung Wook	M	8	Seoul	7.0	500	1:32	1:2
V9-8421	Chai OK Sun	M	Unk	Seoul	6.5	130	1:128	0
				Japanese				
V9-3182	Sugiyama, Takayuki	M	4	Tokyo Prefecture	7.5	40,000	1:128	1:8
V9-3626	Kono, Yosuko	F	10	Tokyo Prefecture	7.2	800	1:32	0
V9-3702	Kojima, Hideko	F	41	Tokyo Prefecture	7.0	1,000	1:64	1:2
V9-3745	Kurihara, K.	F	8	Tokyo Prefecture	7.5	320	1:64	1:2
V9-3806	Tanaka, Fusako	F	29	Tokyo Prefecture	7.3	320	1:64	0
V9-3901	Hayashi, Kyoko	F	8	Aichi Prefecture	7.6	800	1:64	0
V9-3902	Kawahara, Shunji	M	8	Aichi Prefecture	7.5	1,300	1:64	1:2
V9-3903	Kondo, Yoshimasa	M	24	Aichi Prefecture	7.1	320	1:64	0
V9-4358	Hashizume, K.	M	12	Tokyo Prefecture	6.9	1,000	1:64	0
V9-4399	Horiuchi, Masatomo	M	14	Yamanashi Prefecture	7.0	100	1:32	1:2
V9-4661	Hirano, Ikuko	F	29	Tokyo Prefecture	7.4	800	1:32	1:2
V9-4791	Higaki, Kiyoji	M	Unk	Osaka Prefecture	-	-	1:64	1:2
V9-4872	Nakano, J.	M	61	Tokyo Prefecture	-	-	1:32	1:4
V9-5074	Nanaka, Ichiro	M	12	Yamanashi Prefecture	-	-	1:64	0

Table V. Immunization Program, Okayama Prefecture

	Initial Series	Stimulating Dose	Total Immunized
1946	14,523		14,523
1947		14,473	14,473
1948	14,207	11,945	26,152
1949	33,214	20,964	54,178

Occurrence of Cases - a. 1946 - Only one case of Japanese B encephalitis was reported, and this occurred in a 41 year old woman.

b. 1947 - Of 72 reported cases, 68 probable cases included 24 instances among children 9 years of age or younger. No cases occurred in the vaccination group. Because of the spotty distribution of cases and distribution of vaccination program only 3 localities were suitable for comparison.

c. 1948 - Of 100 reported cases, 64 probable cases included 23 cases in the 5 to 10 year age group. In 11 localities suitable for comparison 14 cases occurred in unvaccinated controls and 1 case in a vaccinated child.

d. 1949 - Of 118 reported cases, 110 clinically confirmed cases included 28 cases in the age group under study (Fig. 3). Of these 21 were in localities suitable for comparison (Table VI.).

Figure 3

EVALUATION PROGRAM JAPANESE B ENCEPHALITIS VACCINE

WITH LOCATION OF SCHOOLS AND SUSPECTED PATIENTS

OKAYAMA PREFECTURE · 1949

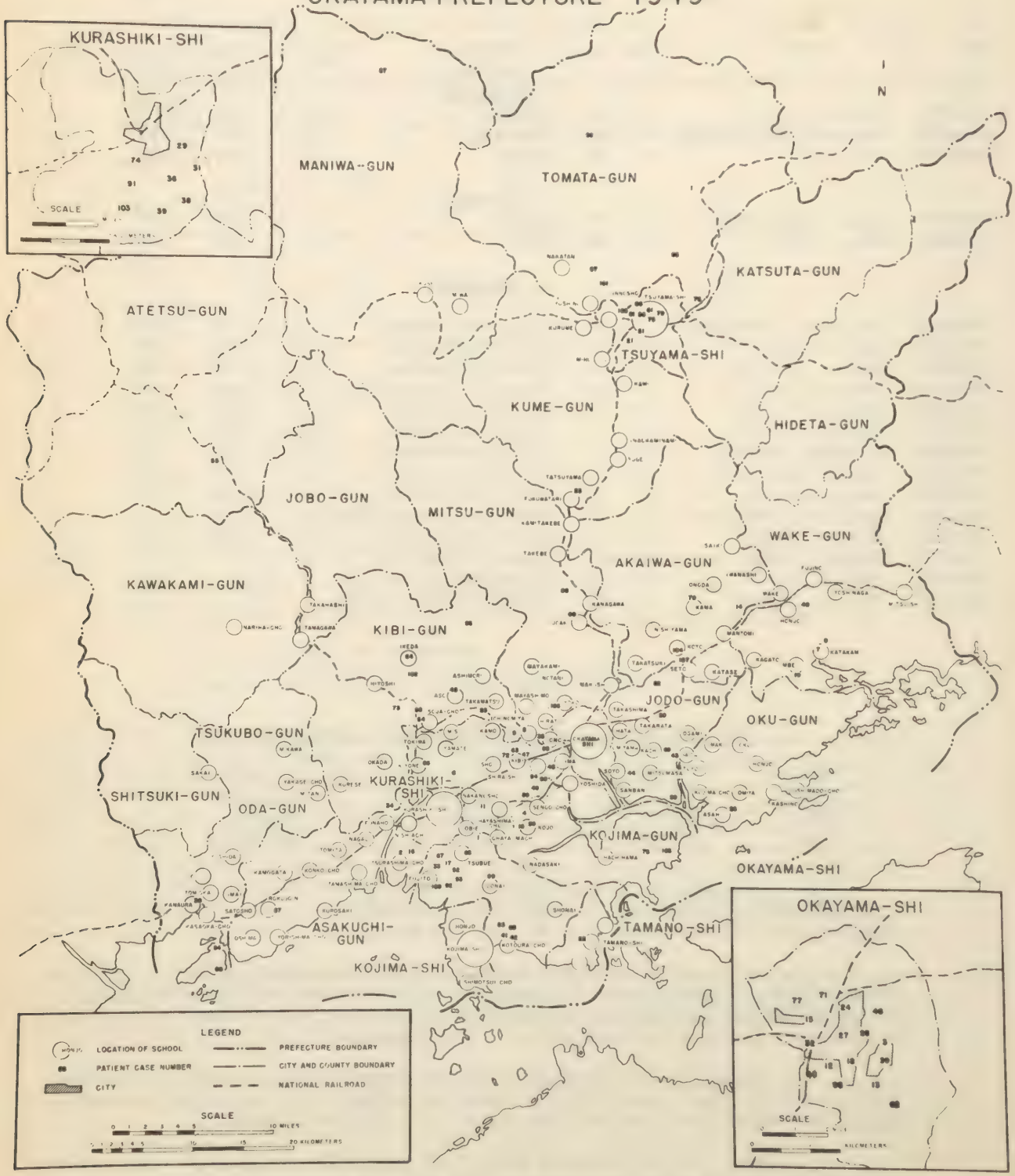


Table VI. Case Occurrence of Japanese B Encephalitis
In Study Group

	Total Pop. 5-10 yrs.	Cases in 5-10 yr. Group	Init. Vacc. 1949	Recall Vacc. 1949	Total Vacc. 1949	Cases In Vacc.	Total Non-Vacc. In 1948	Cases In Non-Vacc.
Okayama City	17,442	2	2188	4940	7128		10,314	2
Kurashiki City	5,872	1	521	2278	2799		3,073	1
Tamano City	6,538	1	633	1928	2561		3,977	1
Kojima Gun								
Fukuda-cho	3,164	4	652		652		2,512	4
Kojo-son	1,170	1	428		428	1	742	
Kotura-cho	2,533	1		559	559	1	1,974	
Tsukubo Gun								
Hayashima-cho	919	1	448	198	646		273	1
Seno-cho	1,215	1	465	280	745		470	1
Kibi-cho	964	1	364	204	568		396	1
Kiyone-son	371	1		157	157		214	1
Mitsu Gun								
Ichinomiya-son	528	1	171	119	290		238	1
Yokoi-son	403	1	200	118	318	1	85	
Asakuchi Gun								
Tsurajima-cho	3,114	1	361	344	705		2,409	1
Rokujoin-cho	874	1	413		413		461	1
Joto Gun								
Saidaiji-cho	1,631	1	649	195	844	1	787	
Wake Gun								
Imbe-cho	822	1	352	93	445		377	1
Kibi-Gun								
Soja-cho	1,300	1	563	306	869		431	1
Total	48,860	21	8408	11,719	20,127	4	28,733	17

Expected cases in vaccinated group at rate of total = 20127/48860x21 = 8.6

Expected cases in vaccinated group at rate of unvaccinated = 20127/28733x17 = 11.9

Actual cases in vaccinated group = 4.

An evaluation similar to that previously performed shows an incidence of 8.6 cases would have been anticipated at the rate of occurrence in the total population of the study group. Similarly 11.9 cases would be expected at the rate of occurrence only in the unvaccinated group. Actually only 4 cases were seen in the vaccinated children.

Instead of limiting the statistical study to those restricted areas in which the scattered cases occurred, a broader analysis utilizing the method of X^2 (chi-squared) may be attempted. The total figures available are shown in Table VII.

The incidence of cases was compared between the non-vaccinated and vaccinated groups as shown in Table VIII. The difference of the incidence of encephalitic cases is significant between the non-vaccinated and the vaccinated (total) groups (Schedule A) and between the non-vaccinated, and the booster plus primary 3 dose groups (Schedule B). However, this significant trend does not persist when the booster (Schedule C) and primary 3 dose groups (Schedule D) are compared individually with non-vaccinated groups. The overall results would indicate the vaccine to be of some prophylactic value as a means of controlling Japanese B encephalitis.

Table VII. Vaccination Program Accomplished in Children in Okayama-Ken*

		Vaccinated				
			Primary Immunization			
	Non-Vaccinated	Booster	3 doses	2 doses	1 dose	Total
No. of individuals in groups indicated	61,124	20,964	33,214	1204	1884	118,390
No. of cases among groups indicated	17	1	2	1	0	21

* Only those data of districts in which population figures were available for both vaccinated and non-vaccinated individuals are included in this chart. This represents the correct control level.

Table VIII. Effectiveness of Vaccination Against Japanese B Encephalitis on Basis of X^2 (chi-squared)

Schedule A	Non-Vaccinated	Vaccinated All Groups	Total	
Non-encephalitic	61,107	57,262	118,369	X^2 = 7.232
Encephalitic	17	4	21	P = <0.01
Total	61,124	57,266	118,390	n = 1

Schedule B	Non-Vaccinated	Vaccinated (Booster plus Primary 3 doses)	Total	
Non-encephalitic	61,107	54,175	115,282	X^2 = 6.983
Encephalitic	17	3	20	P = <0.01
Total	61,124	54,178	115,302	n = 1

Schedule C	Non-Vaccinated	Vaccinated (Booster only)	Total	
Non-encephalitic	61,107	20,963	82,070	X^2 = 2.802
Encephalitic	17	1	18	P = 0.095
Total	61,124	20,964	82,088	n = 1

Schedule D	Non-Vaccinated	Vaccinated (Primary 3 doses only)	Total	
Non-encephalitis	61,107	33,212	94,319	X^2 = 3.217
Encephalitic	17	2	19	P = 0.08
Total	61,124	33,214	94,338	n = 1

Okayama Pre-season Survey - As part of the continued program mentioned above a serologic survey was conducted prior to the 1949 immunization program.

Approximately 200 Japanese primary school children between 6 and 10 years of age living in rural and urban areas of Okayama-Ken received a 1.0 ml subcutaneous dose of chick embryo vaccine, lot #301-B. Blood specimens were obtained immediately prior to and 14 days following vaccine administration. The samples were collected and stored in the usual manner. Pre and post vaccine specimens were tested simultaneously for complement fixing and neutralizing antibodies.

Striking differences were noted in the various groups studied (particularly in complement-fixing antibodies), not only in response to the vaccine but also in the proportion of children in this locality showing positive serologic reactions both prior and subsequent to vaccination (Table IX). Thus, of children from two schools in Okayama-shi, only 6 per cent of 34 in one group (Uchisange) showed positive complement fixation tests 14 days after administration of vaccine, while 56 per cent of 27 individuals in a second group (Seitoku) showed positive complement-fixation tests, a remarkable contrast to previous results. Nine per cent and 18 per cent respectively, of the former group showed positive neutralizing antibodies before and after vaccination. In the latter group (Seitoku), the proportions were 56 per cent and 63 per cent. Differences are also noted between two groups of children in Kurashiki-shi while in the smaller village of Seto-cho, the difference appeared to be somewhat less striking. The results of serologic response with second specimens are shown below in Table IX.

Table IX. Serologic Results with Second Specimen

		Comp. Fixation	Neutralization Index		
		Positive	Positive	Equivocal	Negative
I.	1st Spec. Negative	1/127 - 0.8%	22/127-17.3%	23/127-18.1%	88/127-64.6%
II.	1st Spec. Equivocal	0/12	4/12 -33.3%	6/12 -50.0%	2/12 -16.7%
III.	1st Spec. Positive	39/63 - 62.0%	63/63 -100%	0/63	0/63

When individuals from all areas were regrouped according to the status of demonstrable neutralizing antibodies prior to vaccine administration, a definite pattern of response to vaccination is noted. Of those children showing negative or equivocal neutralization indices before inoculation, relatively few showed positive complement-fixing antibodies 14 days later. A high proportion of children with previously positive neutralization indices showed positive complement fixation titres.

Additional tabular presentation is given in Table XI where results are shown in terms of logs protection (cumulative). Of particular interest is the demonstration that a single dose of vaccine is sufficient to evoke a change in neutralization index from negative to positive in 17.3 per cent of the children studied.

Post Epidemic Surveys of Americans in Tokyo Area - Following the relatively severe JBE epidemic of 1948 an attempt was made to determine the serologic pattern in American military personnel following vaccination and possible inapparent infection.

American Military Personnel, Tokyo Area - Past experience with adult American personnel has indicated that roughly 2 per cent of all such individuals may be expected to develop complement-fixing antibodies in a low titre (1:4), following a series of inoculation with Japanese B encephalitis chick embryo vaccine. In these sera collected in September 1948, from 4 per cent to 15 per cent (average 8 per cent of 340 individuals) of the several groups tested showed a positive complement fixation in serum dilutions of 1:4 and 1:16, highest dilution tested. The results are summarized in Table XII. Members of the 8th Cavalry Regiment from which this survey was made had been utilized for outdoor guard duty in the Tokyo area during the summer of 1948. The "correlation" of positive complement-fixing antibodies with the level of neutralizing antibodies is also shown in Table XII.

Table X. Japanese B Encephalitis Virus Complement Fixation and Neutralizing Antibody Levels in Japanese Children (6-10 years) Before and After Subcutaneous Administration of 1.0 ml Vaccine*

Area	Specimen	Positive Complement Fixation	Neutralization Index Positive	Equivocal	Negative	Total Tested
Okayama-shi (Uchisange)	Pre	0 - 0%	3 - 9%	5 - 15%	26 - 76%	34
	Post	2 - 6%	6 - 18%	12 - 35%	16 - 47%	
Okayama-shi (Seitoku)	Pre	0 - 0%	15 - 56%	2 - 7%	10 - 37%	27
	Post	15 - 56%	17 - 63%	0 - 0%	10 - 37%	
Kurashiki-shi (Otaka)	Pre	0 - 0%	33 - 69%	0 - 0%	15 - 31%	48
	Post	13 - 27%	37 - 77%	4 - 8%	7 - 15%	
Kurashiki-shi (Masu)	Pre	0 - 0%	5 - 23%	0 - 0%	17 - 77%	22
	Post	3 - 14%	6 - 27%	0 - 0%	16 - 73%	
Seto-cho (Takatsuki)	Pre	0 - 0%	4 - 9%	4 - 9%	39 - 83%	47
	Post	3 - 6%	15 - 32%	8 - 17%	24 - 51%	
Seto-cho (Kosai)	Pre	0 - 0%	3 - 13%	1 - 4%	20 - 83%	24
	Post	4 - 17%	8 - 33%	5 - 21%	11 - 46%	

* Lot #301-D, second specimen 14 days after inoculation

Table XI. Results Listed in Table X. Shown in Terms of Logs Protection (Cumulative)

Area	Specimen	Logs Protection (Cumulative) 3.0+	2.0+	1.7+	1.0+	1.0-	Total Tested
Okayama-shi (Uchisange)	Pre	1 - 3%	3 - 9%	3 - 9%	8 - 24%	26 - 76%	34
	Post	5 - 15%	6 - 18%	6 - 18%	18 - 53%	16 - 47%	
Okayama-shi (Seitoku)	Pre	7 - 26%	15 - 56%	15 - 56%	17 - 63%	10 - 37%	27
	Post	15 - 56%	17 - 63%	17 - 63%	17 - 63%	10 - 37%	
Kurashiki-shi (Otaka)	Pre	27 - 56%	32 - 67%	33 - 69%	33 - 69%	15 - 31%	48
	Post	34 - 71%	35 - 73%	37 - 77%	41 - 85%	7 - 15%	
Kurashiki-shi (Masu)	Pre	2 - 9%	5 - 23%	5 - 23%	5 - 23%	17 - 77%	22
	Post	5 - 23%	5 - 23%	6 - 27%	6 - 27%	16 - 73%	
Seto-cho (Takatsuki)	Pre	3 - 6%	4 - 9%	4 - 9%	8 - 17%	39 - 83%	47
	Post	10 - 21%	13 - 28%	15 - 32%	23 - 49%	24 - 51%	
Seto-cho (Kosai)	Pre	2 - 8%	3 - 12%	3 - 12%	4 - 17%	20 - 83%	24
	Post	4 - 17%	6 - 25%	8 - 33%	13 - 54%	11 - 46%	

Survey of Japanese Children in Tokyo Area 1949 - In a study of the serologic pattern in Japanese children in the Tokyo area following the 1948 epidemic a group were bled during January 1949 and subsequently vaccinated with 1.0 ml of Japanese B encephalitis vaccine, lot #229-B (MID, 0.018), and bled again ten days later. Pre and post-vaccine sera were processed in the usual manner and stored in a CO_2 ice chest until all sera could be tested simultaneously.

Table XII. Complement-Fixing and Virus Neutralizing Antibodies Against Virus of Japanese B Encephalitis in Post-1948 Serum Specimens of American Military Personnel Tokyo Area (September 1948)

Group	Complement Fixation Positive	Neutralization Test Logs Protection* 4.0+*	3.0+*	2.0+*	1.7+*	1.0+*	Neg.	Total Tested
FEAF	2/46(4%)	3(8%)	12(30%)	16(40%)	19(48%)	26(65%)	14(35%)	40
8th Cav Regt 1st Cav Div	9/61(15%)	1(2%)	14(23%)	18(30%)	19(31%)	27(44%)	34(56%)	61
5th Cav Regt B Troop 1st Sq	7/100(7%)	1(1%)	23(23%)	35(35%)	37(37%)	55(55%)	44(44%)	99
Shiroi AB	9/133(7%)	2(2%)	12(9%)	21(16%)	29(22%)	35(27%)	98(73%)	131
Total	27/340(8%)	7(2%)	61(18%)	90(27%)	104(31%)	143(43%)	188(57%)	331

* Totals are cumulative from left to right.

Levels of Neutralizing Antibodies of all Sera with Positive Complement Fixation Reactions							
All	5(18%)	18(67%)	3(11%)	0	1(4%)	0	27
Levels of Neutralizing Antibodies of all Sera with Negative Complement Fixation Reactions but with One Log or More Protection in Neutralization							
All*	2(2%)	36(31%)	26(22%)	14(12%)	38(33%)	0	116

In 71 cases, paired sera were available for complete testing; the following results are based only on these cases. Forty of 71 pre-booster specimens were negative for complement fixing and virus neutralizing antibodies. Ten days later the results listed below were found:

Comp. Fix.	Neut. Ind.	
Neg.	Neg.	19/40 (47.5%)
Neg.	Equiv.	11/40 (27.5%)
Neg.	Pos.	9/40 (22.5%)
Pos.	Pos.	1/40 (2.5%)

Thus, only one of 40 pairs showed positive complement-fixing antibodies after administration of a single dose of vaccine (1:16, 3+): neutralization index $> 10,000$). Nearly one fourth became positive for neutralizing antibodies mostly in a low positive range (median 130). Interpretation of neutralization index rise of this magnitude is difficult in the absence of control tests with children of comparable ages living in non-endemic areas. Another one fourth of the entire group showed an increase in their neutralization index to the equivocal range, while nearly 50 per cent of the total remained negative.

In 6 instances, initial complement-fixation reactions were negative and initial neutralization indices were equivocal. Ten days following vaccinations the results listed below were found.

Comp. Fix.	Neut. Ind.	
Neg.	Neg.	3/6 (50%)
Neg.	Equiv.	1/6 (17%)
Neg.	Pos.	2/6 (33%)
Pos.	Pos.	0/6 (0%)

Where complement fixation was initially negative and neutralization index positive (24 cases), positive complement-fixing antibodies appeared following vaccination in 23 of 24 sera (96%). In a single instance, complement-fixing titre remained negative and the neutralization index became equivocal.

In one case, both the complement-fixation and neutralization index were positive prior to vaccination. The complement-fixing titre increased from 1:8 (2+) to >1:16 (4+), while neutralization index showed a slight drop from >10,000 to >8,000. The results with paired specimens are shown in Table XIII.

Table XIII. Paired Specimens - Tokyo Survey

	Age	Positive Sept. 1948	Positive January, 1949	Total	
Daito-Ku	0-4	1/8	9/14	10/22	(45%)
	5-9	2/20	11/22	13/42	(31%)
	10-14	9/17	-	9/17	(53%)
Kita-Tama (Jindai-Mura)	0-4	5/10	0/3	5/13	(38%)
	5-9	8/14	4/16	12/30	(40%)
	10-14	13/19	4/12	17/29	(57%)

On a part of the group of children mentioned above, the results of neutralization tests conducted in 1946 were available and are included in Table XIV. In addition, various other age groups represented on the 1946 survey were re-examined (Chuo-Ku and Jindai-Mura).

So far as is known, no clinical cases of Japanese B encephalitis occurred in any member of these groups. The data noted above also permits some expansion of the general epidemiological picture in Tokyo following the 1948 epidemic.

When no reference was made to the age factor, the following results were noted:

Table XIV. Post-Epidemic Surveys, Japanese Children - Tokyo Area

		Neutralization Index		
		Positive	Equivocal	Negative
Chuo-Ku	1946	37/71 (52%)	0/71 (0%)	34/71 (48%)
	1949	43/71 (61%)	0/71 (8%)	22/71 (31%)
Jindai-Mura	1946	42/74 (57%)	1/74 (1%)	31/74 (42%)
	1949	49/74 (66%)	3/74 (4%)	22/74 (30%)

Of 37 individuals positive in 1946 from Chuo-Ku, 2 (5%) have fallen off to an equivocal level in 1949, while 35 (95%) remained positive. Of 34 individuals negative in 1946, 8 (24%) were positive, 4 (12%) are equivocal and 22 (65%) remained negative in 1949. All of 42 individuals from Jindai-Mura "positive" in 1946 remained positive in 1949. One case "equivocal" in 1946 was negative in 1948. Of 31 individuals "negative" in 1946, 7 (23%) were positive, 3 (10%) were equivocal and 21 (68%) were negative respectively in 1949.

		Chuo-Ku Neutralization Index		
		Neg. 1946	Equiv. 1946	Pos. 1946
Neg.	1949	22/34 (55%)	0/34	0/37 (0%)
Equiv.	1949	4/34 (12%)	-	2/37 (5%)
Pos.	1949	8/34 (24%)	-	35/37 (95%)

		Jindai-Mura Neutralization Index		
		Neg. 1946	Equiv. 1946	Pos. 1946
Neg.	1949	21/31 (68%)	1/1	0/42 (0%)
Equiv.	1949	3/31 (10%)	0/1	0/42 (0%)
Pos.	1949	7/31 (23%)	0/1	42/42 (100%)

Table XV. Comparison of Japanese Children 1946 and 1949*

Area	Age	1946 Neut. Index Pos.	Equiv.	Neg.	1949 Complement Fixation Spec.	Positive	1949 Neutralization Index Pos.	Equiv.	Neg.
Jindai Mura	0-4	0/3	1/3	2/3	Pre Vac	0/3	0/3	1/3	2/3
					Post Vac	0/3	1/3	2/3	0/3
	5-9	0/12	0/12	12/12	Pre Vac	0/12	3/12(25%)	1/12(8%)	8/12(67%)
					Post Vac	3/12(25%)	6/12(50%)	2/12(17%)	4/12(33%)
	10-14	1/7	0/7	6/7	Pre Vac	0/7	1/7(14%)	0/7	6/7(86%)
					Post Vac	0/7	1/7(14%)	2/7(29%)	4/7(57%)
Chuo-Ku	0-4	0/3	0/3	3/3	Pre Vac	0/3	0/3	0/3	3/3
					Post Vac	0/3	2/3	1/3	0/3
	5-9	1/9(11%)	0/9	8/9(89%)	Pre Vac	0/9	2/9(22%)	2/9(22%)	5/9(56%)
					Post Vac	2/9(22%)	4/9(44%)	3/9(33%)	2/9(22%)
	10-14	6/13(46%)	0/13	7/13(54%)	Pre Vac	0/13	9/13(69%)	1/13(8%)	3/13(23%)
					Post Vac	9/13(69%)	10/13(77%)	1/13(8%)	2/13(15%)
Daito-Ku	0-4	-	-	-	Pre Vac	0/4	2/4	1/4	1/4
					Post Vac	2/4	2/4	0/4	2/4
	5-9				Pre Vac	2/20(10%)	9/20(45%)	0/20	11/20(55%)
					Post Vac	9/20(45%)	10/20(50%)	2/20(10%)	8/20(40%)

* Age groups based on American age at time of bleeding. Summary based only on results with individuals from whom both pre and post vaccination specimens were obtained.

Serological Survey in Hokkaido - In September and October 1949 an opportunity presented for a serum survey of Hokkaido. This northernmost island of Japan has long been recognized as relatively free of the annual onslaught of JBE in horses and humans. The epidemic of 1948, however, was reflected by an annual rate of 0.2 per 100,000. Information was desired as to whether there has been an inapparent dissemination of the virus during that year. A total of 192 sera were collected from four areas: (1) Wakkanai, (2) Mombetsu, (3) Asahigawa, and (4) Obihiio. Blood was collected, serum separated and frozen, and specimens subjected to complement-fixation and neutralization tests.

Complement-fixation showed one four-plus reaction in a 1:4 dilution, one anticomplementary serum, and one non-specific reaction to antigen and normal mouse brain in 1:4 dilution. The remaining 189 were negative. Results of neutralization tests are shown by age group in Table XV. When compared with results obtained in 1946 in a similar survey in Sapporo (southern Hokkaido) no increased dissemination of virus in 1948 appears to have occurred (Table XVI). Further breakdown by geographic location suggests no limitation of the disease to any particular portion of the island.

Table XVI. Neutralization Indices in Hokkaido

Age Groups	Hokkaido 1949		Sapporo 1949	
0-4	0/1	0%	1/38	3%
5-9	0/16	0%	2/40	5%
10-14	1/58	2%	0/40	0%
15-19	0/26	0%	2/42	5%
20-39	11/51	21%	2/20	10%
40-59	4/24	17%	4/19	20%
60-	0/2	0%	7/41	17%
Total	16/178	5.8%	18/240	8.6%

(The numerator denotes sera with neutralization index of 50 and higher. The denominator denotes number of sera tested).

Japanese B Encephalitis (Equine Encephalomyelitis) - Summary of 1948 Epizootic - Because of the extended period of time required to obtain epidemiological data together with the added interference imposed by the Japanese language barrier, this tabulation of the Japanese Equine encephalomyelitis epizootic as it occurred in 1948 has been delayed.

This epizootic was the largest ever reported, there being a total of 3678 cases as compared to 1209 during the summer of 1947.

The outstanding point of significance in the 1948 epizootic was extension of the disease in epizootic form to Hokkaido (Fig.4). Previously, this island had been considered as a non-epidemic and non-epizootic area, with only sporadic cases being previously reported. This present reported incidence leaves little doubt that the virus of Japanese B encephalitis has a distribution throughout Japan.

Probably the greater number of susceptibles in those prefectures which previously (1947) had a low attack rate account for the extremely high morbidity rate (greater than 50 cases per 10,000 equine population) observed in Aomori, Iwate, Tochigi and Ibaraki Prefectures. A converse relationship was observed in those prefectures with high morbidity rates in 1947 (Chiba, Gifu, Tottori and Kochi). There was an exception in that Yamagata Prefecture reported a morbidity rate greater than 50 cases per 10,000 equine population for both 1947 and 1948. The five prefectures of Aichi, Osaka, Hyogo, Mie and Nara reported no cases during the past two years of observation. It is felt that these areas do not enjoy a specific peculiarity of animal non-infectivity.

Table XVII gives a tabulation of the total number of equine cases reported in 1948.

Table XVII. Equine Epizootic of 1948 (Total for Japan)

	Mares	Males	Castrates	Total
	708,710	129,343	276,171	1,114,315
Cases 1948	1,857	864	931	3,678*
Morbidity rate per 10,000	26.2	66.75	33.71	32.17
Deaths (including animals destroyed)	740	394	370	1,516**
Fatality per cent (Deaths/cases x 100)	39.85	45.5	39.74	41.22

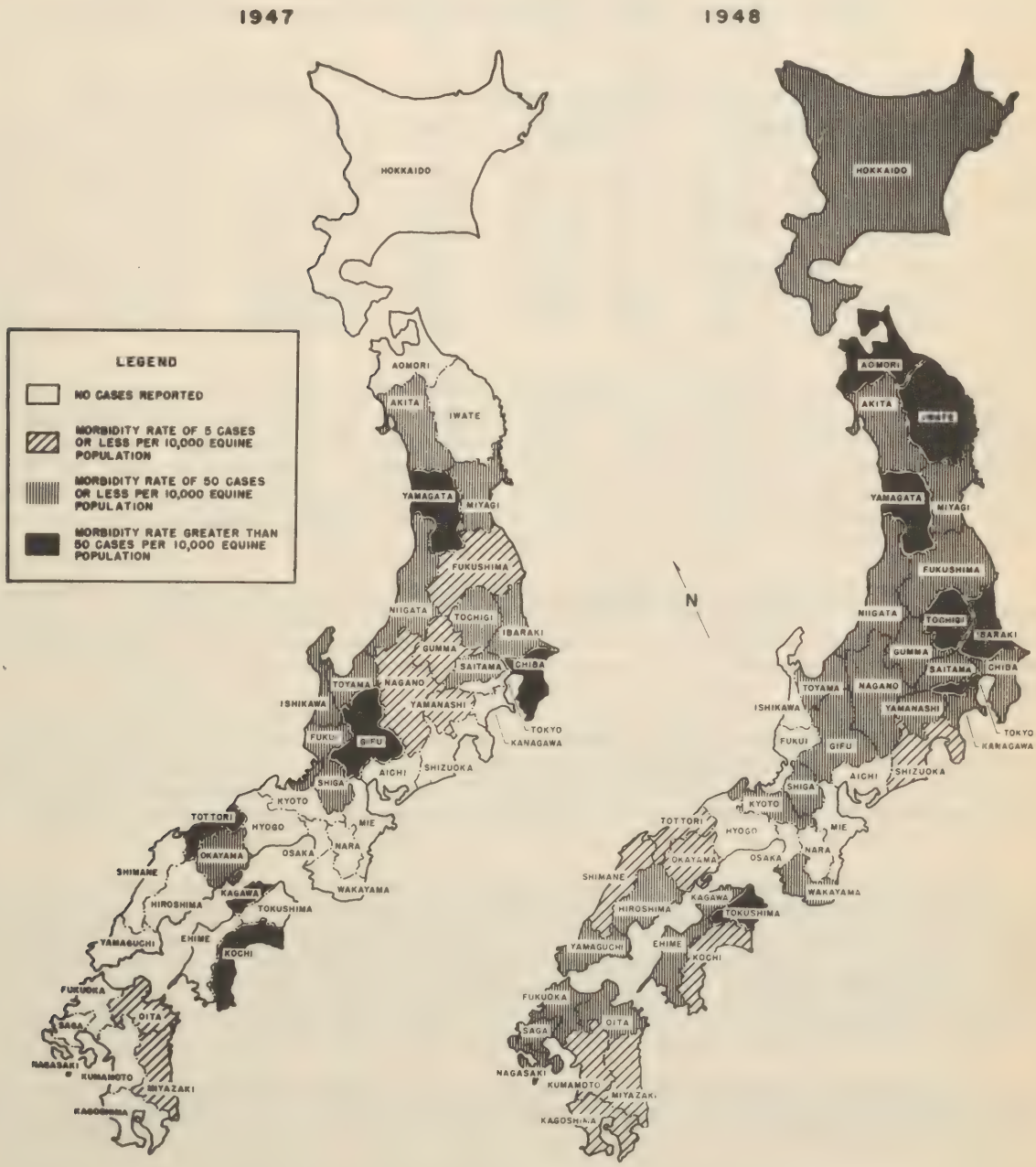
* Includes 26 cases without data as to sex
** Includes 12 cases without data as to sex

Recalculation of the above, based on 20 prefectures that reported more than 20 cases each provide essentially similar comparitive rates.

Figure 4

JAPANESE EQUINE ENCEPHALOMYELITIS

DISTRIBUTION AND DEGREE OF INCIDENCE



Fuchu and Nakayama Epizootic of 1948 and Survey Data - During the 1948 season, 37 equines from these two race track areas in Tokyo exhibited signs of encephalomyelitis and were positive by complement-fixation and neutralization tests. (From animals sacrificed, isolates of Japanese B encephalitis virus were obtained). Significant rises in titre on either test were rare. Blood samples were collected from 195 apparently normal equines stabled in the same areas and from 150 human beings, also apparently normal, who lived in the same areas (many were engaged as caretakers, etc.). Serologic examinations are summarized below.

Table XVIII. Summary of Serological Tests for Japanese B Encephalitis Virus in Normal Humans and Equines in the Fuchu and Nakayama Areas.

Group		Complement Fixation Test			Neutralization Test			
		Positive	Negative	Total	Positive	Equivocal	Negative	Total
Horse	Fuchu	126 (86.9%)	19 (13.1%)	145	138 (95.2%)	6 (4.1%)	1 (0.7%)	145
	Nakayama	42 (84.0%)	8 (16.0%)	50	47 (94.0%)	2 (4.0%)	1 (2.0%)	50
	Total	168 (86.7%)	27 (13.3%)	195	185 (94.9%)	8 (4.1%)	2 (1.0%)	195
Humans	Fuchu	2 (3.0%)	65 (97.0%)	67	49 (73.2%)	4 (6.0%)	14 (20.8%)	67
	Nakayama	0 (0%)	83 (100%)	83	54 (65.1%)	7 (8.4%)	22 (26.5%)	83
	Total	2 (1.3%)	148 (98.7%)	150	103 (68.7%)	11 (7.3%)	36 (24.0%)	150

The difference in pattern of complement-fixing antibodies in human beings and equines in the same area is notable. Numerous explanations can be advanced but a difference in exposures (i.e., virus dosage) appears most reasonable, since the human in relation to the animal lives a relatively sheltered life. Some support for this is found in the comparative figures for neutralizing antibodies since these probably represent a cumulative effort over a period of years in the case of the human beings. These figures are not significantly different for human beings in other areas of Tokyo.

Yoyogi Stabled Horses, 1949 Survey - Twenty-four young (1 to 2 years old) horses were obtained from the northern part of Hokkaido (Kitami) for experimental purposes. Previously this island had been considered as non-epidemic and non-epizootic until 1948 when an epizootic occurred. However, these horses were secured from an area in Hokkaido where no cases were reported.

Horses were stabled at Yoyogi (in Tokyo) primarily as a susceptible group to detect the initial viral injection (inapparent infection) should an outbreak occur during the 1949 season. The tabulated results in Table XIX show that all the horses during the months of July and August were negative for complement-fixing and neutralization antibodies except horse No. 20, which had an initial neutralization index of 400 in July.

Without any clinical manifestations, a majority of horses developed low titre complement fixing antibodies during September; this is to be expected in inapparent infections. The complement-fixing antibody titres during October, November and December showed a diminishing pattern. Those that remained positive exhibited a very low titre. In addition a large number of positives changed to a negative category. The complement-fixing antibodies for Japanese B virus in equines are of a transitory nature, their period of duration extending through three or perhaps four months.

All the horses developed neutralizing antibodies during September with rising titres during October, the peak being reached during November with a few neutralization indices approaching the 100,000 mark.

This data suggests that both complement-fixing and neutralizing antibodies begin to develop about the same time. Complement-fixing antibodies begin to wane while neutralizing antibodies are still increasing, hence it is important that these serological tests be run simultaneously if they are to be used as an epidemiological instrument.

Table XIX. Neutralization Indices and Complement Fixation Titres of Yoyogi Stabled Horses

Horse No.	Dates Horses Were Bled: July 8 N.T.	July 8 C-F	Aug 11 N.T.	Aug 11 C-F	Aug 25 N.T.	Aug 25 C-F	Sep 30 N.T.	Sep 30 C-F	Oct 25 N.T.	Oct 25 C-F	Nov. 23 N.T.	Nov. 23 C-F	Dec. 20 N.T.	Dec. 20 C-F
1	3	0	4	0			13,000	1:16(3/)	13,000	1:16(3/)	20,000	0	5,000	0
2	3	0	2	0			6,000	0	6,300	0	80,000	0	8,000	0
3	5	0	3	0			630	0	10,000	0	32,000	0	8,000	0
4	2	0	5	0			2,500	0	16,000	0	2,500	0	5,000	0
5	13	0	5	0			2,500	1:8(3/)	13,000	1:4 (4/)	10,000	0	8,000	0
6	6	0	10	0			2,500	1:4(4/)	13,000	1:4 (4/)	25,000	0	5,000	0
7	3	0	2	0			8,000	1:8 (4/)	13,000	1:4 (4/)	32,000	1:4	10,000	0
8	3	0	5	0			8,000	1:8 (4/)	4,000	1:8 (3/)	50,000	1:8	5,000	1:4
9	2	0	3	0			800	0	5,000	0	16,000	0	13,000	0
10	6	0	2	0			8,000	1:8 (4/)	32,000	1:4 (4/)	130,000	0	10,000	1:4
11	8	0	2	0			8,000	1:4 (4/)	25,000	1:4(3/)	80,000	0	8,000	0
12	5	0	5	0			100	0	2,500	0	800	0	500	0
13	13	0	6	0			1,300	0	25,000	0	13,000	0	3,200	0
14	6	0	2	0			8,000	1:4 (4/)	13,000	1:4 (4/)	32,000	1:4	4,000	1:4
15	6	0	2	0			13,000	1:4 (3/)	13,000	0	100,000	0	10,000	0
16	40	A.C.	2	0			13,000	1:8 (4/)	13,000	1:8 (4/)	63,000	0	3,200	1:2
17	6	0			32	0	13,000	1:4 (4/)	40,000	1:4 (4/)	25,000	0	5,000	0
18	6	0			8	0	400	0	8,000	0	13,000	0	8,000	0
19	10	0			20	0	2,000	0	13,000	0	20,000	0	8,000	0
20	400	0			80	0	4,000	1:8 (3/)	20,000	1:4 (4/)	63,000	1:4	5,000	1:4
21	10	0			13	0	5,000	1:4 (3/)	63,000	0	80,000	0	10,000	0
22	6	0			20	0	13,000	1:8 (3/)	32,000	1:8 (4/)	160,000	1:4	autopsied	
23	6	0			40	0	630	0	5,000	0	6,300	0	5,000	0
24	16	0			20	0	6,300	1:4 (3/)	8,000	0	63,000	0	16,000	0

* Bac #6 6,300
Bac #7 20,000

* Positive Cont. (Last year's experimental horses)

The difference in pattern of complement-fixing and neutralizing antibodies in various individual equines in the same stabled area is noted. The difference in exposure, that is, the virus dosage, appears to be one explanation besides inherent individual variation. It is probable that a positive complement-fixing titre means a recent infection even if sub-clinical. According to this limited group, the usefulness of the neutralization and complement fixation tests as an etiological diagnostic tool in equines is limited.

Mosquito Transmission of Japanese B Encephalitis Virus - Transmission studies with species of mosquitoes found in the Tokyo areas were undertaken beginning in July 1949. These studies were designed to develop techniques for further work on the biological relationship existing between arthropod and Japanese B encephalitis virus. Japanese investigators, many years ago, reported mosquito transmission of the virus (12), transovarian transmission of the virus to progeny of infected females, and survival of the virus over winter in hibernating adults. Investigations have been undertaken to determine if these results can be corroborated.

A summary of the transmission data shows that with 16 lots of Culex tritaeniorhynchus, consisting of 128 mosquitoes, there were 8 transmissions to young mice. Of these 8 transmissions, 7 lots of mosquitoes were infected on blood virus suspensions and one lot on an infected chicken.

Aedes togoi transmission experiments resulted in 2 transmissions. Eight lots of mosquitoes made up of a total of 78 specimens were used. In each instance, as in all the following experiments, the infectious meal was a blood-virus suspension.

Four transmissions were obtained with 21 specimens of Aedes albopictus, pooled in 7 lots. One lot transmitted once, while another lot transmitted three times.

Culex pipiens undoubtedly is a much better vector than these results indicate, as it was poorly adapted to the conditions of the experiments. Of 10 lots of 98 mosquitoes total, one transmission to a young mouse was obtained. C. pipiens fed very reluctantly on mice.

The same statement may be made for Armigeres subalbatus, which failed to transmit. Five lots of a total of 55 specimens were tested.

Transovarian transmission has given only negative results in the preliminary studies made. Recovery of the virus has been attempted by feeding the progeny of infected females on young mice. Three lots totalling 103 specimens of C. tritaeniorhynchus, 4 lots, totalling 28 Culex pipiens and one Aedes albopictus have been tested in this way.

Over 600 specimens of Culex pipiens have been infected and placed in hibernation cages in hope they will survive the winter so they can be tested in the spring for presence of Japanese B encephalitis virus.

Antibody Response and Irradiation - JBE vaccine administration and pre and post season surveys have been performed in conjunction with the laboratory of the Atomic Bomb Casualty Commission at Hiroshima, using individuals exposed and unexposed to the historic irradiation. While laboratory tests have been completed, no opportunity has been found to evaluate the results.

Other Viral Encephalitides - Okinawa Outbreak, Etiology Unknown - During the year, 20 clinical cases have been observed in Okinawa with an undiagnosed and relatively mild condition which clinically appears to be a virus encephalomyelitis.

In general, the symptoms consisted of a moderately severe conjunctivitis, nuchal rigidity, headache, retro-orbital pain, with muscular weakness and/or tenderness. Muscles involved were usually triceps, hamstrings and quadriceps. Most patients were admitted on about the third day of the disease. The initial temperature of 102° to 104.5°F usually fell by the sixth day of the disease and after a 24 to 36 hour interval was frequently followed by a secondary rise to 100° to 101°F. The fever tended to disappear by approximately the 8th to 10th day, leaving in some instances slight residual weakness of muscle groups and mild headache. Spinal fluid examination showed no abnormality or only a slight pleocytosis in the first several days. With the recurrence of fever, the total count in spinal fluid reached its highest levels (during the 7th to 10th days of the disease) following which there was a gradual reduction in cells. Early specimens showed moderate proportions of polymorphonuclears, with shift to predominant lymphs. Total proteins showed a similar rise shortly after the peak of pleocytosis. Sugar was normal to increased (glucose infusions).

Unfortunately, at the time information was received of the occurrence of these cases the "epidemic" had passed. Attempts at inoculation of whole blood and extracts of fecal specimens were negative. A single later case, probably not the same disease, had fresh spinal fluid injected intracerebrally and intraperitoneally into white mice with negative results.

It has been indicated and references are available to suggest this disease has manifested itself on several occasions in the past. These include a report entitled, "Dengue-Like Fever on Okinawa", by Col. Walter B. Martin, MC, who was at the time medical consultant to the Tenth Army, another report entitled, "Clinical Characteristics of Dengue-Like Disease" on Okinawa, which appeared in Medical Bulletin No. 10, Headquarters Island Command, dated 23 July 1945 by Col. James B Stapleton, and report to Preventive Medicine Section, SGO, by Dr. A. B. Sabin on 28 December 1945.

Sabin also described several patients on Okinawa with clinical manifestations not unlike those in the recent outbreak (21). Of special interest is the fact that the tests for Japanese B encephalitis were negative. As is further pointed out, similar outbreaks

have been studied in the Philippines and Japan. During Sabin's stay in Japan in 1946, he noted (21) a number of patients with the syndrome described above, including saddle-back fever. Stools from a number of these patients were sent to Dr. J. R. Paul at New Haven, Connecticut who recovered poliomyelitis virus from two sera and data on the above-mentioned cases have been forwarded to the AMDR&GS for virus studies.

Philippine Infection, Etiology Unknown - In October and November, an unusual infection of some twenty military personnel and dependents were reported. These cases manifested typically a short febrile illness (about 3 days) with nuchal rigidity, headache and pleocytosis. Attempts at virus isolation by the local laboratory were unsuccessful. Information and convalescent sera were submitted to this laboratory. Additional local investigation has been accomplished with more serum and information obtained. This material and data will be forwarded to AMDR&GS. Consideration has been given to the possible relation of this condition to encephalomyocarditis and/or viruses of the Coxsackie group.

Typhus Fever - Complement-fixation tests with sera for diagnosis of typhus fever from Japanese nationals were conducted at the request of the Public Health and Welfare Section, SCAP. Soluble antigens prepared in this laboratory were included in the testing of all specimens. Highly specific antigens, which allow for a differentiation between epidemic and murine typhus, were obtained from the Army Medical Department Research and Graduate School.

On the basis of the number of specimens received this year, both epidemic and murine typhus appeared in relatively small numbers and were scattered throughout Japan proper. The number of specimens received from any given area would not indicate this year to be one in which an unusual incidence of the disease was occurring. The largest number of typhus suspects among Japanese nationals with positive complement fixation tests were reported from Fukushima-Ken, with only 14 cases reported. Murine typhus appeared to be scattered from Hokkaido to Kyushu with the greatest incidence being reported in Nagasaki Prefecture (Table XX).

Complement Fixation Tests of Typhus Fever in Japan, 1949 - A total of 1472 serum specimens were examined for complement-fixing antibodies for typhus fever which represented 262 suspect typhus cases. Of 262 suspect cases, a total of 96 specimens gave positive complement fixation reactions in either one or more specimens taken during convalescence.

For purposes of these tests, a 3 or 4+ complement fixation reaction in a dilution of 1:10 was considered as significant. In addition, in order for serums to be specific for epidemic or murine typhus, it had to produce a titre for the homologous antigen over that of the heterologous antigen by an 8-fold or more difference. Otherwise it was considered as type undetermined. Thirty-nine of this group were positive for epidemic typhus antigen and 21 were positive for murine. Thirty-seven were positive for both epidemic and murine; hence, could not be identified by difference in titre. A total of 166 were negative. Those showing positive reactions with soluble antigens and not with epidemic or murine are reported as negative. Of the total group, 13 sera were anticomplementary.

In contrast to the small number of cases occurring this year, the results of complement fixation tests for typhus fever in Japan during 1948 are more revealing. Approximately 30 per cent of the sera tested this year proved to be serologically positive for typhus complement fixing antibodies, as compared to 1948's fifty per cent positive sera reactions. In view of the fact that in 1948 there were 80 type undetermined reactions, together with 37 in 1949 which showed identical titres for both epidemic and murine antigens, the presence of an unidentified type of typhus must be borne in mind.

Scrub Typhus - Introduction - Following the demonstration of clinical cases in the Gotemba area in 1948, epidemiological considerations (14) pointed clearly to the fact that the series of typhus cases arose from exposure to vectors of the disease.

Table XX. Japanese Typhus Fever Suspects with Positive Complement Fixation Tests

Prefecture	No. of Suspect Cases	Positive Epidemic	Positive Murine	Type Undetermined	Total Pos.	Total Neg.	No. of Sera
Aichi	11	3	1	1	5	6	21
Aomori	2	-	-	-	-	2	2
Chiba	1	1	-	-	1	-	1
Fukui	1	-	-	-	-	1	1
Fukuoka	3	-	1	-	1	2	3
Fukushima	17	12	1	1	14	3	24
Gifu	2	-	-	-	-	2	4
Hiroshima	3	-	1	-	1	2	3
Hokkaido	9	3	-	-	3	6	13
Kanagawa	7	1	-	2	3	4	7
Kumamoto	12	-	1	-	1	11	12
Mie	1	-	-	-	-	1	1
Hyogo	-	-	-	-	-	-	-
Miyagi	18	11	-	-	11	7	25
Nagasaki	37	1	5	6	12	25	58
Nagano	7	-	2	1	3	4	8
Nara	26	1	1	2	4	22	38
Osaka	22	2	2	6	10	12	27
Saga	3	-	-	1	1	2	5
Saitama	5	-	-	3	3	2	10
Shizuoka	1	-	-	1	1	-	1
Tokyo	41	-	2	5	7	34	56
Tottori	26	1	3	7	11	15	42
Wakayama	-	-	-	-	-	-	-
Yamagata	2	-	-	-	-	2	2
Total	262	39	21	37	96	166	368

In order to determine the presence of vectors and principle reservoirs of this disease, in 1949 a spot survey was established in the Gotemba area.

Mite Collection from Fuji Manouver Area - During the period 26 to 29 September 1949, field search for chiggers was conducted by trapping field rodents, placing penned white rats in various locations, running ground litter through a Berlese funnel, and attempting to make boot and card collections of free living chiggers. Of the 28 white rats placed in 3 localities, one rat had 3, another only one chigger. All other efforts to find chiggers by use of the above-mentioned methods were negative. Twelve field mice,(Apodemus speciosis) and 4 shrews (Urotrices telpoides) were trapped. Four of the field mice yielded chiggers, the largest number on any one mouse being approximately 20. All 4 shrews were negative for chiggers.

Serum Surveys for Scrub Typhus - In view of the fact that the Gotemba area was to be used again in 1949 for military maneuvers, it was deemed necessary to provide individual protection against mites, in order to avoid infection with scrub typhus. Impregnation of clothing with a miticide was recommended and used. Various units used the area at different times.

This laboratory planned to draw paired blood specimens from maneuvering troops during the summer and fall. By utilization of the Weil-Felix (OXK) agglutination test on these sera, it was hoped to obtain indirect evidence of the efficacy of the miticide measures which were practiced in that particular area.

The usual procedure consisted of arranging with the units concerned for the bleeding of approximately one hundred men. These individuals were selected as representative of

the different maneuver areas and phases of duty. The initial blood was obtained within the first week of arrival in the area while the second specimen was obtained following the close of maneuvers. The interval of time between the specimens ranged from two to five weeks. In some instances only terminal specimens were obtained. All samples were processed in the usual manner and the sera stored in a dry ice chest until the Weil-Felix agglutination test could be performed. All sera were tested in pairs, or the simple specimens in groups of 50.

All of the paired sera specimens tested proved to be negative for proteus OXK agglutination. There were, however, a number of insignificant agglutination titres in the low range from 1:40 to 1:80. Past experience has shown that a titre of at least 2+ agglutination in a 1:160 dilution is necessary to get beyond the fortuitous range of non-specific agglutination.

Three clinically suspect cases treated with chloromycetin could not be confirmed by laboratory means.

Chemotherapeutic Effect of Chloromycetin against Scrub Typhus in Niigata Prefecture, 1949 - On the basis of laboratory evidence and clinical trials, chloromycetin was found to be markedly effective against scrub typhus (15 and 16) and epidemic typhus (17 and 18). It was also found to be of some prophylactic value for certain of the large viruses of the psittacosis-lymphogranuloma group (19).

This year's field trial was carried out under the immediate supervision of Dr. M. Kitaoka of the Japanese National Institute of Health.

It is a well known fact that scrub typhus in Japan has a more severe clinical course associated with a higher mortality rate than the disease in Malaya and Formosa. During the 1949 (July and August) scrub typhus outbreak in Niigata Prefecture, 10 cases were selected and hospitalized for treatment with chloromycetin. Diagnosis of scrub typhus was made as follows: a detailed clinical history was taken with special emphasis placed on those individuals actually working in infected areas, and on the presence of a mite bite associated with an initial lesion and local adenopathy, followed by fever and appearance of a rash. An eschar was invariably found together with adenopathy at the site of the bite.

Laboratory studies were performed to confirm the diagnosis. The rickettsemia before and after treatment was determined by mouse inoculation with patient's blood taken at daily intervals. In addition, blood specimens were drawn daily to determine the Weil-Felix reaction during the course of the disease.

The treatment was begun between the 2nd and 9th days of the disease. Each patient was given an initial oral dose of chloromycetin calculated as 50 mgm per kg. of body weight, followed by a 0.2 to 0.3 gm. oral dose every 3 hours for varying periods of time (figures 5 and 6). In all cases there was a rapid fall in pulse rate and body temperature within an average of 3.2 days after the initial dose during any stage of the disease. The duration of an elevated temperature greater than 38°C from the onset of illness gave a mean of 8.5 days. All cases were discharged from the hospital on an average of 11.8 days after onset of the disease. The conjunctivitis disappeared within one to 1½ days, along with the rash within 2½ days following the initial dose.

No evidence of appreciable toxicity attributable to the drug was obtained. Among ten cases, two patients exhibited very mild complications in which bronchitis and otitis were observed. There were no deaths among the group treated with chloromycetin.

Concomitantly, 17 cases of scrub typhus were treated with PABA. The beneficial effect of PABA in treatment of these patients is summarized in Table XXI. The initial oral dose was 4.0 to 8.0 gm of PABA followed by 2.0 gm. every two hours day and night unless the blood concentration reaches a level in excess of 50 mg. per 100 ml. (Figs. 7, 8, 9 and 10).

Figure 5

CHEMOTHERAPEUTIC EFFECT OF CHLOROMYCETIN[NO. 1] AGAINST SCRUB TYPHUS

NIIGATA 1949

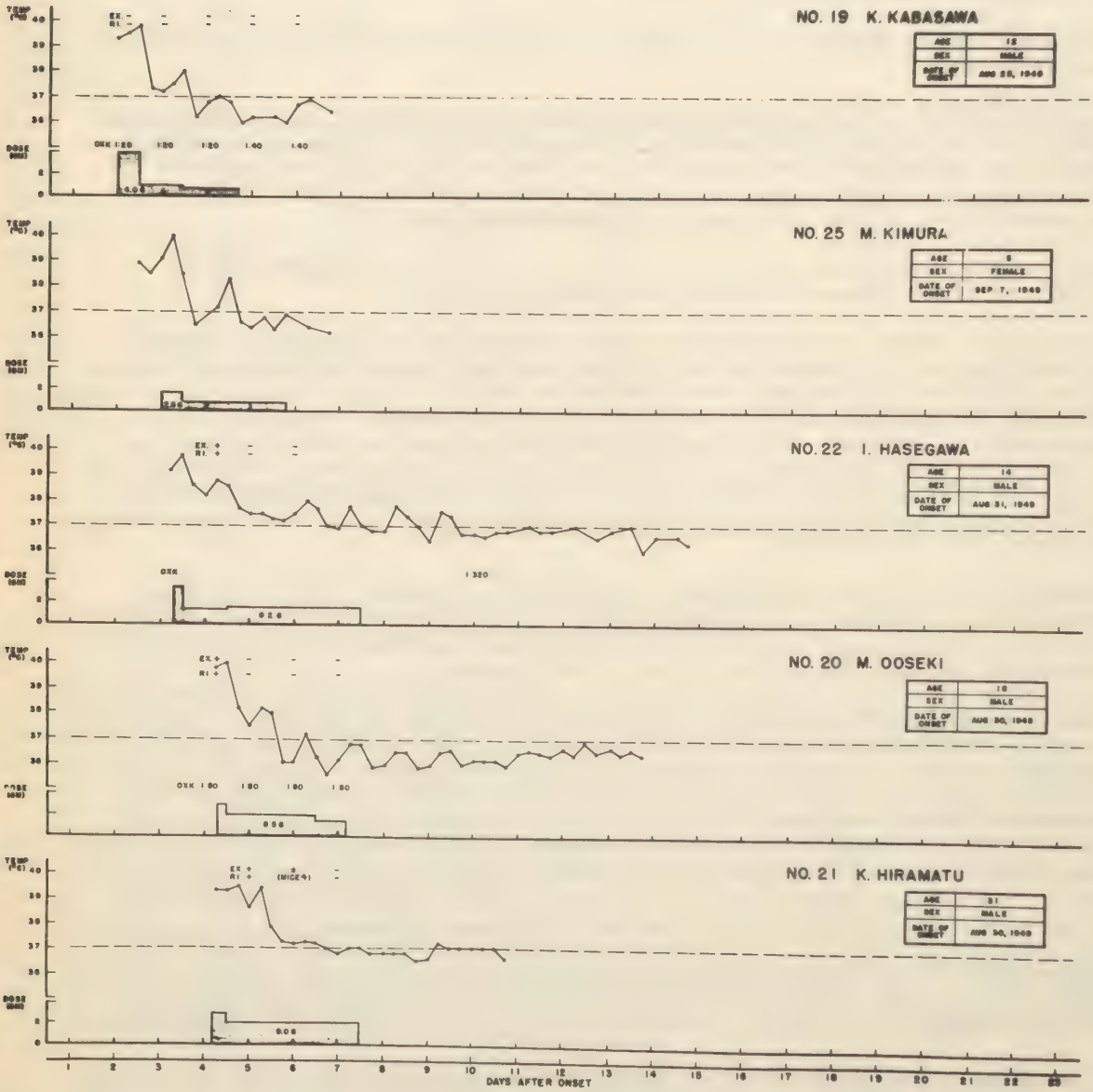


Figure 6

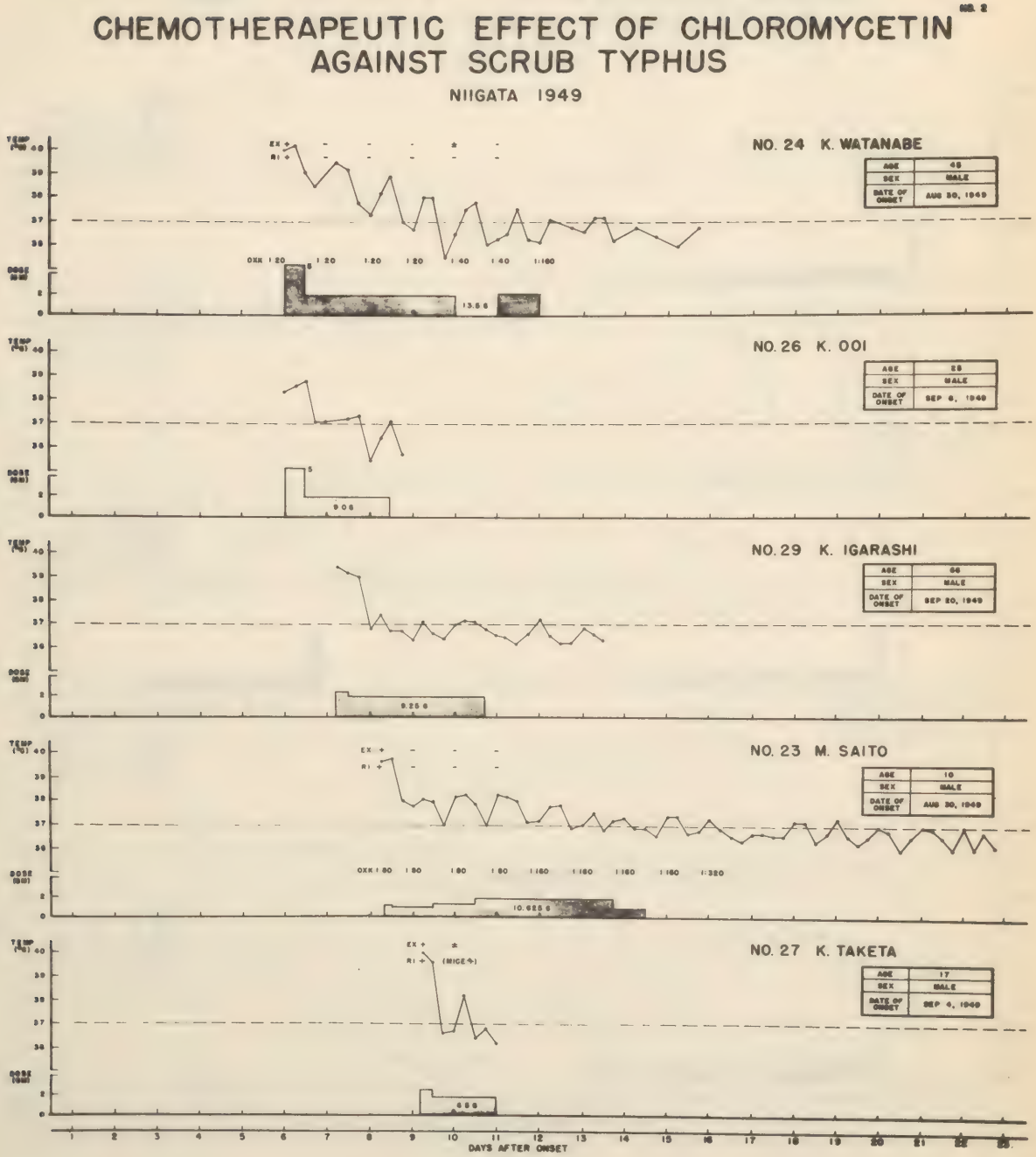

CHEMOTHERAPEUTIC EFFECT OF CHLOROMYCETIN NO. 2
AGAINST SCRUB TYPHUS
NIIGATA 1949
NO. 24 K. WATANABE
NO. 26 K. OOI
NO. 29 K. IGARASHI
NO. 23 M. SAITO
NO. 27 K. TAKETA
DAYS AFTER ONSET

Figure 7

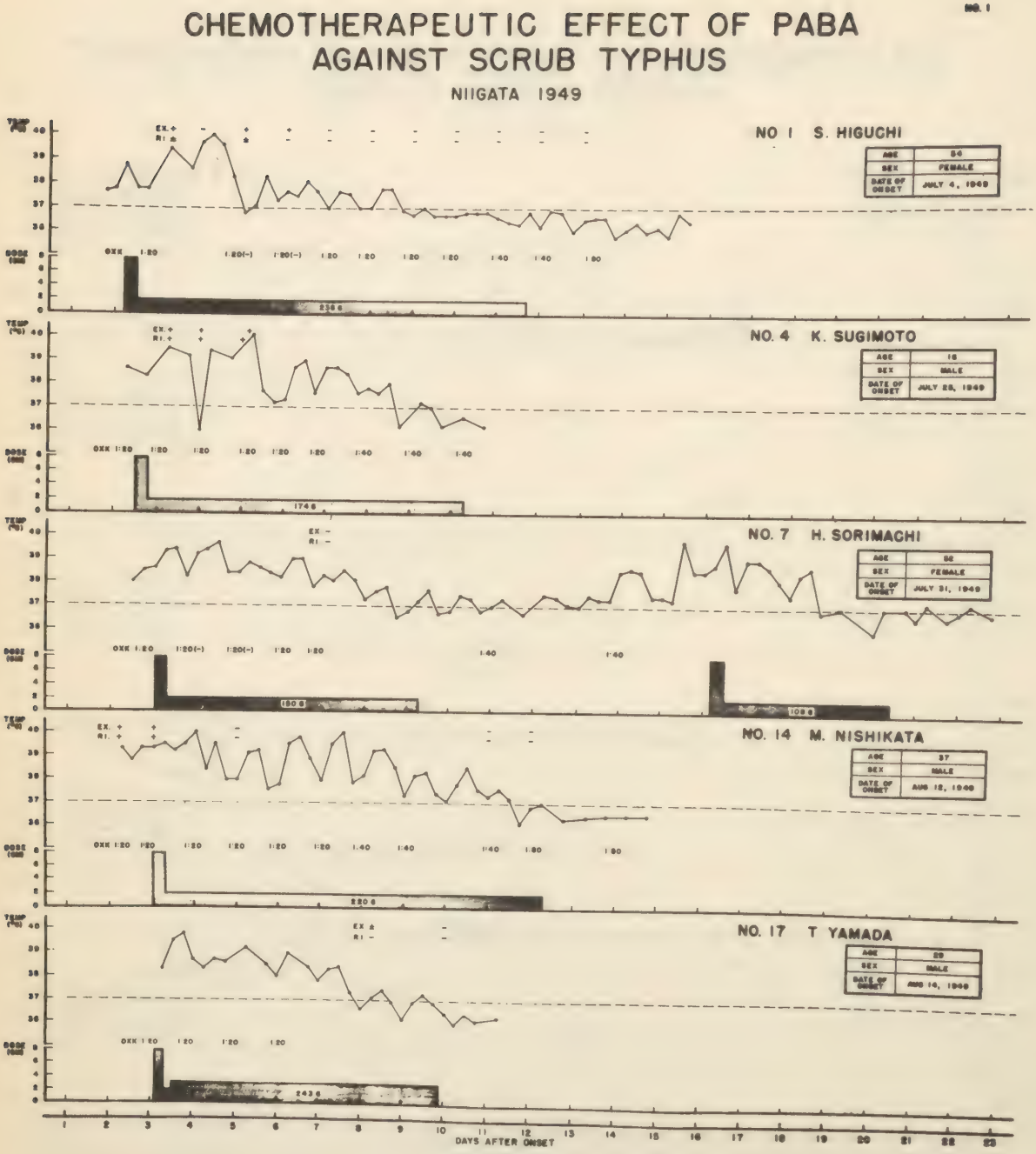

CHEMOTHERAPEUTIC EFFECT OF PABA
AGAINST SCRUB TYPHUS
NIIGATA 1949
NO. 1
NO 1 S. HIGUCHI
NO. 4 K. SUGIMOTO
NO. 7 H. SORIMACHI
NO. 14 M. NISHIKATA
NO. 17 T YAMADA
DAYS AFTER ONSET

Figure 8

NO. 2

CHEMOTHERAPEUTIC EFFECT OF PABA AGAINST SCRUB TYPHUS

NIIGATA 1949

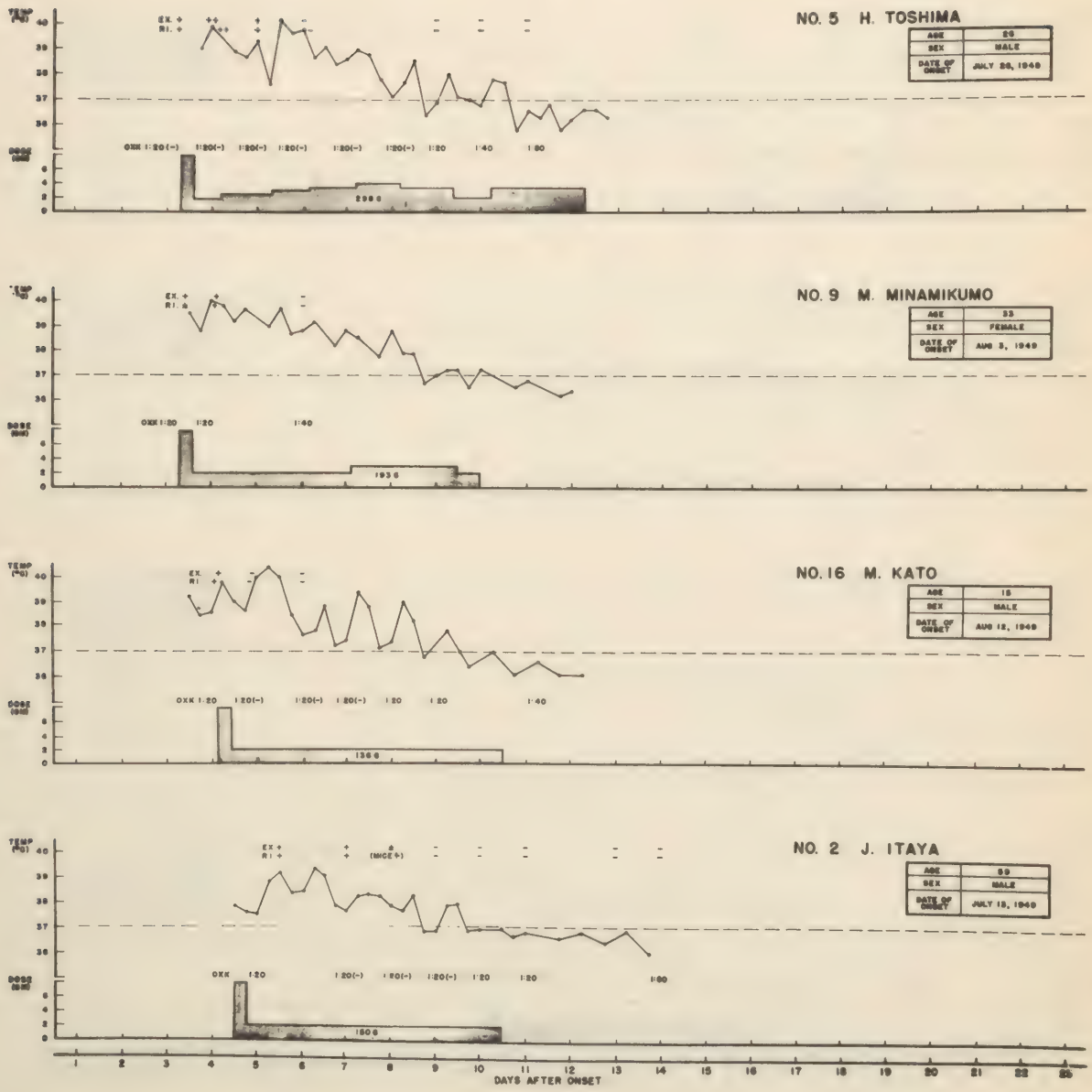


Figure 9

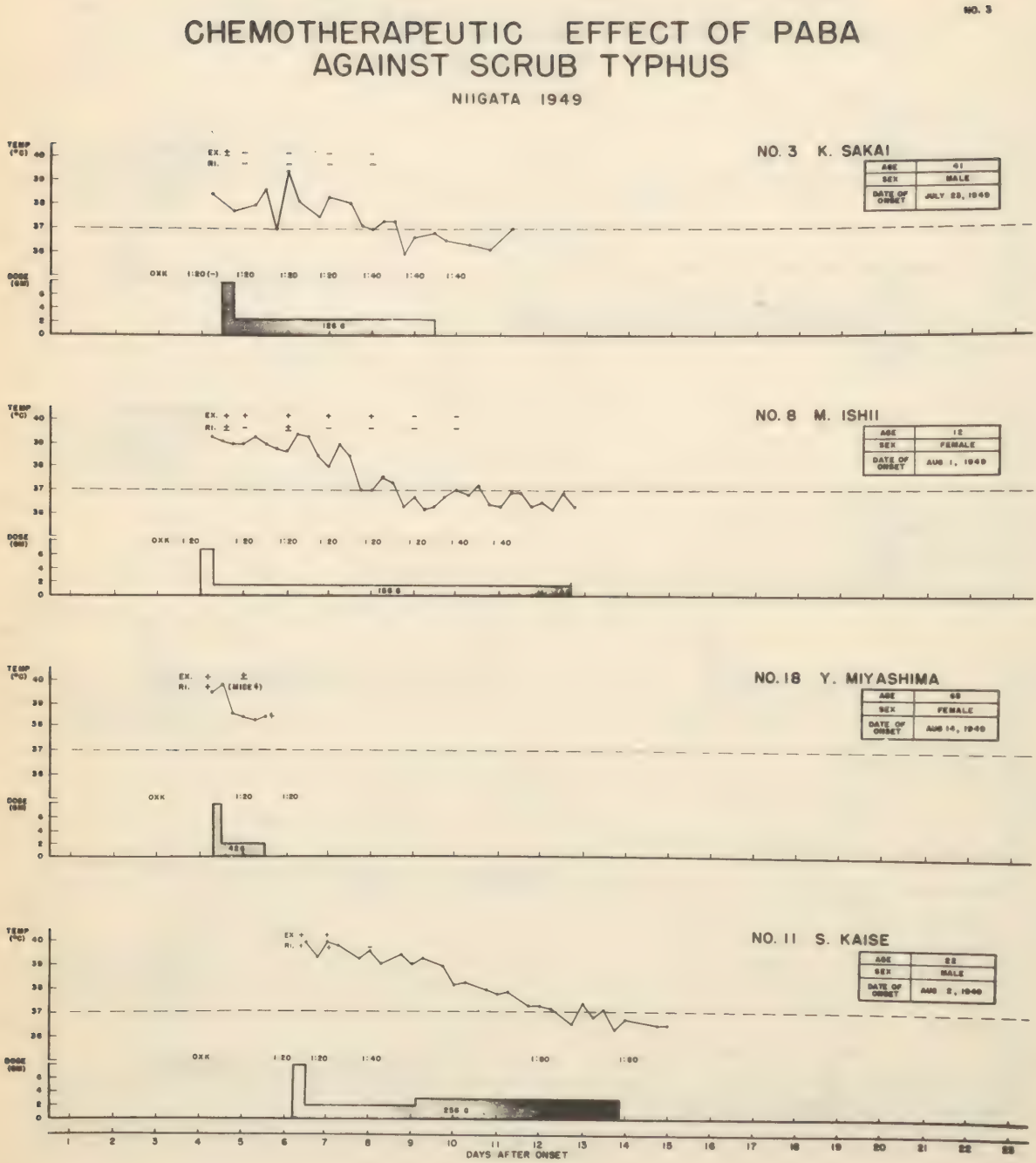

NO. 3
CHEMOTHERAPEUTIC EFFECT OF PABA
AGAINST SCRUB TYPHUS
NIIGATA 1949
NO. 3 K. SAKAI
NO. 8 M. ISHII
NO. 18 Y. MIYASHIMA
NO. 11 S. KAISE
TEMP (°C)
DOSE (GM)
OXK
DAYS AFTER ONSET

Figure 10

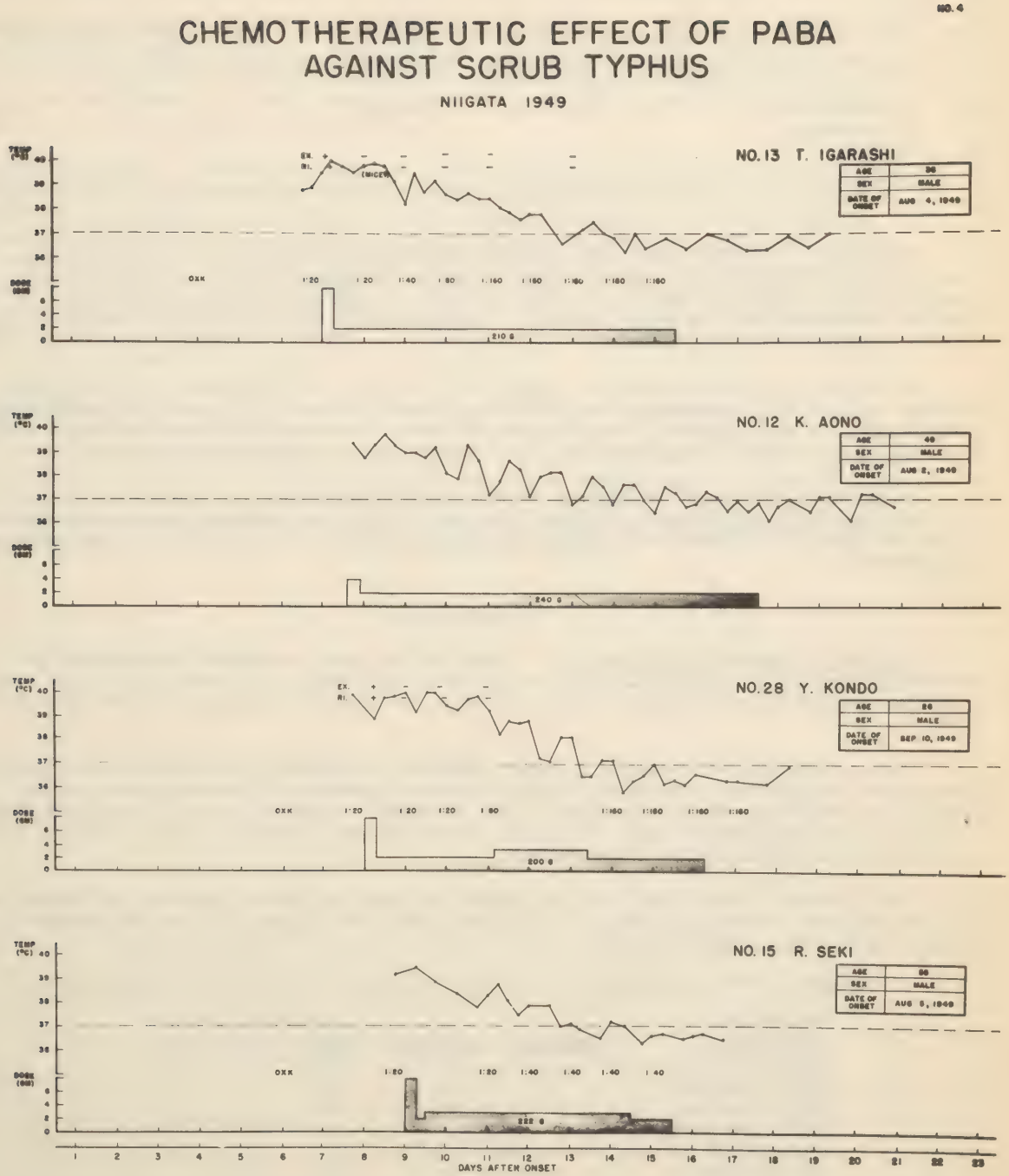

NO. 4
CHEMOTHERAPEUTIC EFFECT OF PABA
AGAINST SCRUB TYPHUS
NIIGATA 1949
NO. 13 T. IGARASHI
NO. 12 K. AONO
NO. 28 Y. KONDO
NO. 15 R. SEKI
DAYS AFTER ONSET

Table XXI. Chemotherapeutic Effect of Chloromycetin and PABA Against Scrub Typhus in Niigata Prefecture, 1949

No. of Cases	Group	Febrile Duration Greater than 38°C. After Initial Treatment	Febrile Duration Greater than 38°C. After Onset of Disease	Days Hospitalization Required	Rash	Conjunctivitis	Complication	No. of Relapse	Death Rate
10	Chloromycetin	3.2 days	8.5 days	11.8 days	2.5 days	1-1.5 days	0	0	0
17	PABA	6.3 days	11.1 days	14.2 days	4.2 days	4.1 days	0	1	1/17
19	Controls (1947) 12 recovered) 7 (died)		14.1 days	25.1 days	-	-	-	-	= 7/19 36.7%

Among the 17 treated cases, one died and another had a relapse. The 15 remaining cases had an average of 11.1 days of fever following treatment with PABA. The rash and conjunctivitis disappeared within 4.2 and 4.1 days after initial dose respectively. It took an average of 14.2 days from onset of disease until discharge from the hospital. All patients had a very stormy course.

If we use as a control level 19 cases (1947) without such treatment, the chemotherapeutic effect of chloromycetin is quite significant. Seven of 19 cases died (mortality rate 36.7%) and 12 recovered. The duration of fever after onset of disease averaged 14.1 days while the number of days required for discharge from hospital was 25.1 days.

It appears from the above-limited results that chloromycetin is quite effective in the treatment of scrub typhus. Although the use of PABA displayed some beneficial results after the onset of disease, there seems no doubt that its value is small in comparison with chloromycetin.

Miscellaneous

Serologic Diagnosis of Influenza - A total of 89 paired specimens representing 149 influenza suspect cases were examined for influenza antibodies. A total of 76 pairs gave negative results while 13 showed significant rise in antibody titre against either "A" or "B".

During the month of December, a flurry of upper respiratory infections among Japanese were reported in Kagawa Prefecture, with a clinical diagnosis of influenza being made. The Public Health and Welfare Section of SCAP arranged for the bleeding of these patients for acute and convalescent sera. Of the 21 paired specimens received, three showed a 4-fold or more rise in titre against "Lee" strain.

The following inset shows the distribution of positive paired specimens as determined by the influenza virus agglutination inhibition test. FM-1 appears to be the predominating strain encountered during the year.

Influenza Virus Agglutination - Inhibition Tests (Positive Paired Specimens)

Prefecture or Hospital	Influenza Strains Japanese	American	FM-1	PR 8	Lee
376th Sta Hosp		2	2		1
155th Sta Hosp		1	1		
361st Sta Hosp		2	2		
128th Sta Hosp		3	2	2	
385th Disp		1	1		
5th Sta Hosp		1	1	1	
Kagawa Pref	3				3
Total	3	10	9	3	4

Equine Isolations - During the past season 12 equine cases were autopsied and specimens forwarded to this laboratory for neurotropic studies.

Three specimens were so badly decomposed that no attempt was made to recover the etiological agent; the remaining 8 specimens were negative on animal inoculations. The sole isolate recovered this season was from a 2 year old equine in Saitama Prefecture which was identified as a rabies virus (V9-3580).

Rabies Isolations - In a recapitulation of rabies cases for the year 1949, it was found that 27 specimens had been submitted to this laboratory for identification. Eleven of the 27 canine and feline specimens exhibited Negri inclusion bodies and presence was confirmed by animal inoculations. Ten of the 11 positive specimens came from the Tokyo-Yokohama-Saitama area.

Avian Isolations - During the months of July, August and September, 157 groups of nestlings (swallows, sparrows, martins) were brought to the laboratory for neurotropic isolation attempts (possible reservoir hosts of Japanese B encephalitis). Virus isolation procedures were attempted on a composite sample of liver and spleen, which were made into a 20 per cent suspension and inoculated into young swiss mice intraperitoneally and intracerebrally. If mice failed to show characteristic symptoms of disease on original inoculation, two consecutive blind passages were carried out before reporting negative results. No neurotropic agent was isolated during the season from approximately 400 birds.

Diagnostic Agents - All antigens produced in 1949 were prepared according to the method described by Espana et al (2). A total of 3533 ml. of Japanese B encephalitis benzene extracted antigen was prepared by this section for use in complement fixation tests. In addition, 2159 ml. of normal mouse brain antigen, together with 773, 91 and 30 mls. of St. Louis, SLE, WEE and EEE respectively, were produced. A total of 423 ml. (all types) of hyperimmune sera were produced as diagnostic agents for the complement fixation and virus neutralization tests.

ENTOMOLOGY SECTION

With the assignment of a competent Entomologist to this laboratory, a definite defect in the organization was corrected by the activation of a section qualified to conduct a careful study of the suspect vector of Japanese B Encephalitis - the mosquito. Personnel were also sent from the Hooper Foundation of the University of California to assist in this joint mosquito study. For administrative reasons this group functioned as a subsection of the Virus and Rickettsial Section. The degree of overlap on the specific problems of Japanese B Encephalitis and Scrub Typhus is evident in the reports of each of these sections. Certain phases of the work primarily entomologic are dealt with here. For other portions dealing with the actual handling of virus, that section should be consulted.

Inasmuch as the dividing line between Routine Special Projects and Research is practically imperceptible in this field, the usual subdivision of work performed followed in this report up to this point cannot be effected.

A simple grouping and addition gives the following summation of activities in general.

Table I. Summary of Collections and Identifications - Entomology Subsection

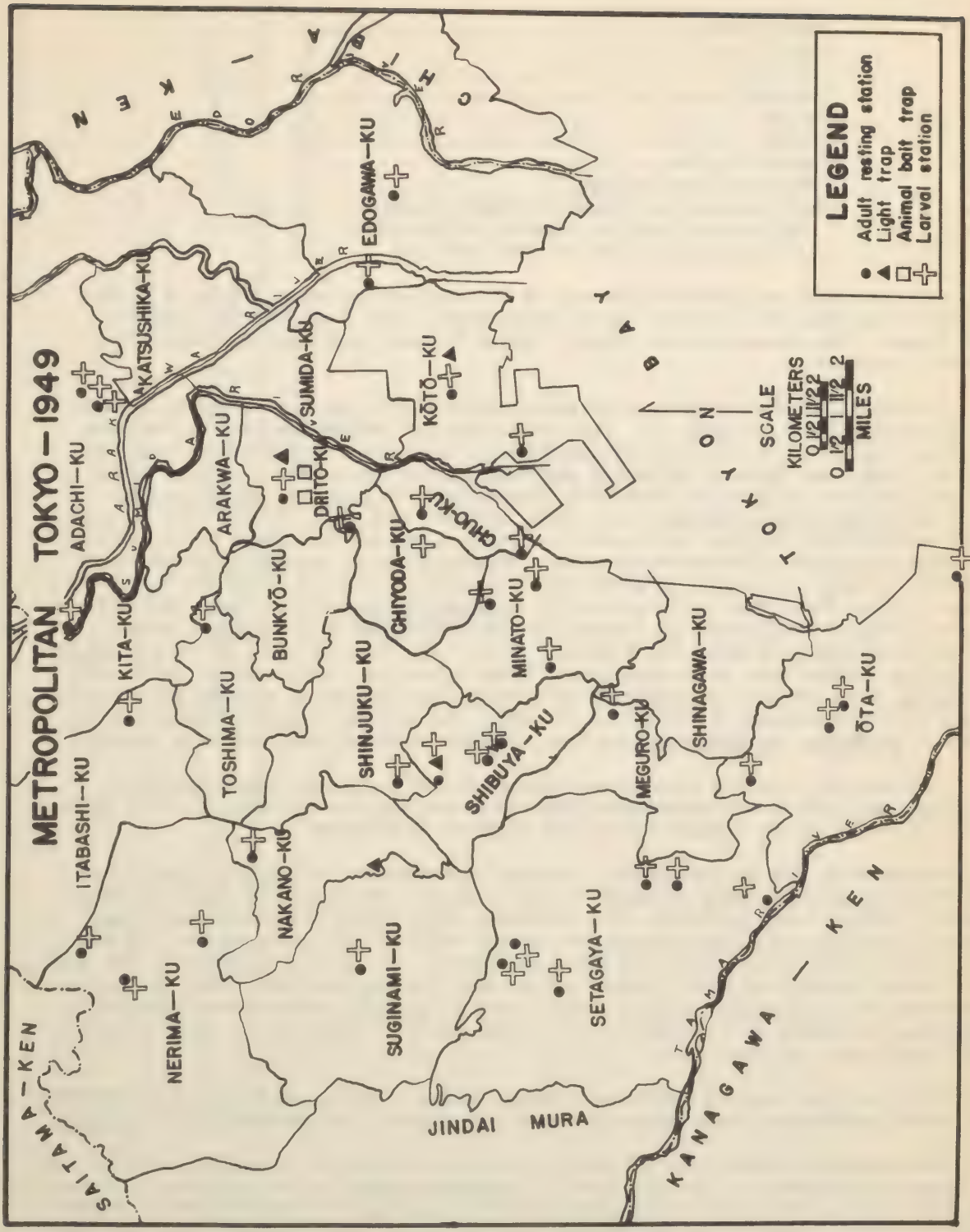
Adult Mosquito Identifications	109,899
Mosquito Larvae Collections	1,338
Larval chiggers	39
Rodents	29
Total	111,305

Mosquito Population Studies in Relation to Japanese B Encephalitis - Introduction - During the period 29 May to 16 October 1949 studies were undertaken in Tokyo, Japan, to determine the relationship of various mosquito species to the transmission of Japanese B Encephalitis. The objectives of this work were twofold: (1) to obtain large numbers of living mosquito adults for virus isolation attempts, and (2) to determine whether or not any correlation could be shown between species population levels and the onset of epidemics and epizootics of the disease. It was hoped that accomplishment of these objectives would give more precise information on the mosquito as a vector and would indicate whether or not a basis for the prediction of epidemics could be determined.

A total of 109,649 adult mosquitoes were taken alive in the field, anesthetized and identified in the laboratory. Of this number, 82,625 mosquitoes were sealed in glass tubes and then frozen on dry ice until virus isolation tests could be conducted. The frozen mosquitoes were divided in half by alternate lots, half being sent to the Hooper Foundation, University of California, for virus isolation tests, the remaining lots being retained by the 406th Medical General Laboratory for similar tests. Reports on the results of these virus isolation attempts will be made at a later date.

Mosquito adults were taken using the following methods: Adult resting station collections, light trap collections, and animal bait trap collections. Every effort was made to maintain established methods of collecting throughout the period of study. Some day and night biting collections were made on humans but the data are too limited to be of value. In addition, 1,338 larval collections were made throughout the study, usually in conjunction with adult resting station collections. Over 1,700 blood smears from engorged female adults were prepared during this work, to be tested by precipitin reaction for information on host tropisms. Results of this phase of the survey will also be reported at a later date.

Adult Resting Station Collections - Thirty-six adult resting stations were selected from areas scattered throughout Tokyo (Map 1). Commencing with 29 May and extending through 15 October, these stations were visited once weekly and an effort was made


METROPOLITAN TOKYO — 1949
LEGEND
Adult resting station
Light trap
Animal bait trap
Larval station
SCALE
KILOMETERS
MILES
ADACHI—KU
KATSUSHIKA—KU
EDOGAWA—KU
KITA—KU
ARAKWA—KU
SUMIDA—KU
KŌTŌ—KU
BUNKYŌ—KU
CHIYODA—KU
CHUO—KU
MINATO—KU
SHINJUKU—KU
SHIBUYA—KU
MEGURO—KU
SHINAGAWA—KU
ŌTA—KU
TOSHIMA—KU
NAKANO—KU
NERIMA—KU
SUGINAMI—KU
SETAGAYA—KU
ITABASHI—KU
JINDAI MURA
SAITAMA—KEN
KANAGAWA—KEN
TOKYO BAY
406 Med. Gen. Lab.

MAP 1.

to completely collect all mosquito adults. Some of the stations were located in urban areas, others in rural areas, some in areas occupied by the Occupation Forces, but the majority were located in strictly Japanese areas. These adult resting stations were selected from a variety of habitats, including:

Horse stables	5
Dairy barns	2
Pig pens	1
Chicken houses	2
Houses	5
Sheds	5
Subway stations	2
Caves and bomb shelters	7
Miscellaneous and mixed (culverts, bombed-out houses, bridges, hunting blinds, and combinations of two or more habitats)	7

Any given station included from one to five collecting units. Thus, a horse stable station may have included one to three barns, or a house station three to five houses. The classification "sheds" refers to small buildings unoccupied by humans or animals.

Tabulation of adult resting station collections from the various types of habitats are summarized in Table II. This table provides collection data on the more important species, a total of 1,081 specimens of miscellaneous species having been omitted from these tables. In animal shelters such as horse stables, dairy barns and pig pens the two species taken most frequently were Culex tritaeniorhynchus Giles and Culex pipiens pallens Coq. These data are misleading in that the collectors would collect from adjacent sheds when the animal shelters were found to be negative for mosquito adults, thus indicating large numbers of Culex pipiens from animal shelters, a large proportion of which should actually be listed under the "shed" category. It was found that collection of C. tritaeniorhynchus from animal shelters consisted almost exclusively of females, while collections of C. pipiens yielded almost equal number of males and females. While very large proportions of C. tritaeniorhynchus females were found engorged in these situations, much smaller numbers of C. pipiens were found to be engorged, indicating that the former species was found in animal habitats because of its desire to feed. It was also found that large proportions of Anopheles hyrcanus sinensis Wied. females in animal shelters were engorged.

Collections of adults from chicken houses showed a decided preponderance of Culex pipiens, 99% of all mosquitoes taken in this situation belonging to this species. Thirty per cent of the females taken were found to be engorged.

Collections from houses showed a distinct predominance of Culex pipiens. Over 98% of all mosquitoes taken belonged to this species. These data again may be misleading, since biting collection data available (vide infra) indicates that Culex tritaeniorhynchus and Culex pipiens bite man in roughly equal proportions. It must be stressed that the data given in Table II indicate the resting tropisms of the various species perhaps to a far greater extent than feeding tropisms. While a greater percentage of C. tritaeniorhynchus females than C. pipiens females were found to be engorged, the number of specimens of the former species taken was too small to allow such significant conclusions.

The remaining types of habitats - sheds, subway stations, caves and bomb shelters, and miscellaneous and mixed stations - also show a great preponderance of C. pipiens.

The numerical superiority of Culex pipiens from all types of resting stations can be largely attributed to the fact that C. tritaeniorhynchus probably rests in natural habitats such as foliage and vegetation, while C. pipiens is known to rest in structures or caves. Other collecting methods, such as light trap or animal bait trap collecting, presented a very different picture of the mosquito population, indicating early in the survey that only by the analysis of several collecting methods could an unbiased and complete picture of mosquito populations be attained.

Table II. Mosquito Collections From Adult Resting Stations by Type Habitat

Habitat	Anopheles hyrcanus Total M&F	%F.	%F. Engorg.	Armigeres subalbatus Total M&F	%F.	%F. Engorg.	Aedes vexans nip. Total M&F	%F.	%F. Engorg.	Culex pipiens pallens Total M&F	%F.	%F. Engorg.	C. tritaeniorhynchus Total M&F	%F.	%F. Engorg.	Totals
Horse stables	272	100	58	21	90	37	204	100	42	3103	47	8	3048	100	55	6648
Dairy barns	889	99	43	645	94	54	98	100	63	5532	55	18	4199	88	75	11163
Pig pens	49	100	47	2	100	0	7	100	43	743	67	20	1641	100	83	2442
Chicken houses	0	0	0	0	0	0	0	0	0	2324	80	30	24	100	29	2348
Houses	2	100	50	16	88	29	1	100	0	4887	59	16	45	93	29	4951
Subway stations	3	100	33	0	0	0	8	100	0	537	56	13	57	91	21	605
Sheds	5	60	0	147	63	0	27	78	0	4812	46	8	113	65	1	5104
Caves and bomb shelt.	22	77	24	6	100	0	6	83	0	18289	36	5	68	88	8	18391
Misc. and Mised sta.	88	94	63	7	86	17	18	89	6	18271	35	8	457	82	49	18841
Totals	1330	99	47	844	88	45	369	98	42	58298	43	11	9652	93	67	70493

An analysis of adult resting stations by location in rural or urban areas produces some interesting results. Of the thirty-six stations utilized, eighteen, or exactly half, were found to be located in rural areas, the other half being located in predominantly urban areas. This distribution of stations was fortuitous, no effort having been made to locate stations in this manner. Rural stations are defined as those stations located in areas where agriculture predominates. Table III indicates the difference in the catches of various mosquito species from the two types of areas. Collections of Anopheles hyrcanus sinensis Wied., Armigeres subalbatus (Coq.), Aedes vexans nipponii (Theob.) and Culex tritaeniorhynchus Giles predominated greatly in stations located in rural areas, while there was a very slight preponderance of Culex pipiens pallens Coq. in urban stations. However in both urban and rural resting station collections C. pipiens was the predominant species. The larger catches of A. hyrcanus, A. subalbatus, A. vexans and C. tritaeniorhynchus in rural stations may be accounted for by the occurrence of most of the animal resting stations in the rural areas, while the slight predominance of C. pipiens in urban areas may be attributed to a larger number of cave or bomb shelters in the urban areas.

Table III. Adult Resting Station Collections from 18 Urban and 18 Rural Stations

Species	Urban Areas No.	%	Rural Areas No.	%	Total No.
Anopheles hyrcanus	12	1	1286	99	1298
Armigeres subalbatus	7	1	837	99	844
Aedes vexans	51	14	309	86	360
Culex tritaeniorhynchus	524	6	8967	94	9491
Culex pipiens	30343	52	27555	48	57898
Misc. Species	362	35	685	65	1047
Totals	31299	44	39639	56	70938

Weekly collections of mosquito adults from resting stations are summarized in Table IV. It was found that the only species showing a distinct "spiked" population peak was Culex tritaeniorhynchus, this peak occurring during the week beginning July 24 (see Fig. 1). This week also saw the peak for the Anopheles hyrcanus population, but the peak for this species was not as well defined. Similarly, the last week in July also served as the peak week for the total mosquito population, taking all species into consideration. It should be noted that with the exception of Culex tritaeniorhynchus, Anopheles hyrcanus, and Aedes vexans, mosquito species tended to show population curves characterized by plateaus rather than by distinct single peaks.

Information on the relative sexual proportions in adult collections taken from resting stations have been included in Table II. Males and females of Culex pipiens were collected in the approximate ratio of 6:4, while other species showed distinctly greater proportions of females. The ratio of males collected to females generally tended to remain stable during the entire collecting season. Kitaoka, Miura and Ogata (1) reported that during the 1948 collecting season they found periods in which the number of males of Culex pipiens greatly exceeded the number of females of this species. Such periods were interpreted by them as indicating the emergencies of distinct broods of Culex pipiens during the breeding season. While such a conclusion based on their reported data would be justified, our data for the year 1949 indicates no such variation in the male-female ratio. Table V. lists the weekly collections of C. pipiens for both sexes. The largest proportion of male C. pipiens were taken during the week beginning 4 September when males constituted 67% of the catch. The figure is hardly high enough to be interpreted as a brood emergence.

Figure 1.

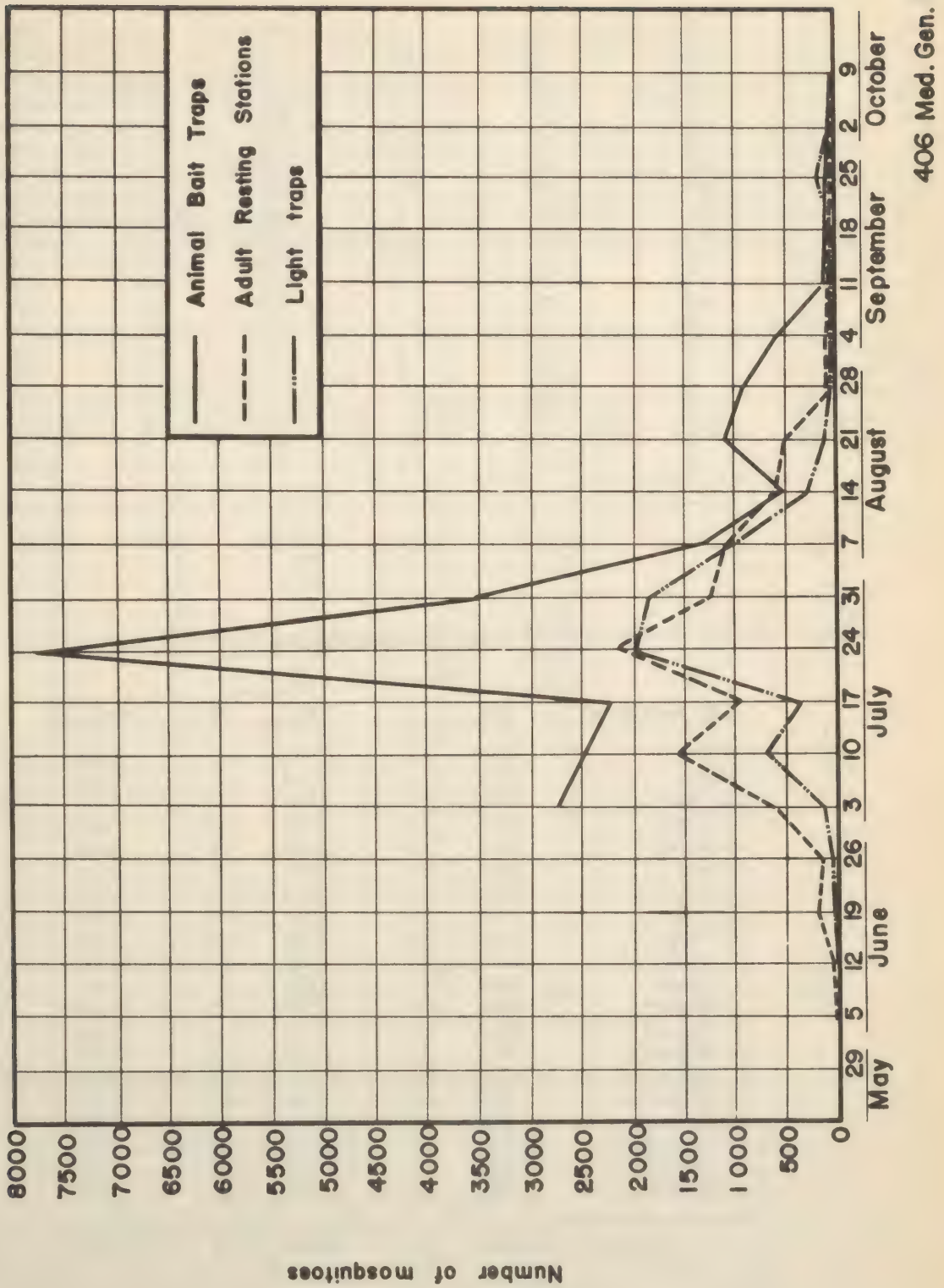

Culex tritaeniorhynchus Adult Collections Tokyo 1949
Animal Bait Traps
Adult Resting Stations
Light traps
Number of mosquitoes
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
29 May
5 12 19 26 June
3 10 17 24 31 July
7 14 21 28 August
4 11 18 25 September
2 9 October
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Table IV. Weekly Collections of Adult Mosquitoes from Resting Stations in 1949

Week beginning	No. Collections	Anopheles hyrcanus sinensis	Armigeres subalbatus	Aedes vexans nipponii	Culex pipiens pallens	Culex tritaeniorhynchus	Misc. Species	Totals
29 May	24	0	0	3	1281	1	9	1294
5 June	36	2	4	5	2338	4	49	2402
12 June	36	0	1	5	4416	29	65	4516
19 June	35	32	24	23	3810	233	84	4206
26 June	36	35	42	85	4696	204	65	5127
3 July	35	72	52	51	3717	650	59	4601
10 July	37	230	34	118	4498	1647	77	6604
17 July	36	244	13	22	2979	949	70	4277
24 July	36	255	20	5	4150	2181	84	6695
31 July	37	194	86	10	4306	1325	75	5996
7 August	35	130	41	12	4486	1100	67	5836
14 August	36	69	20	8	4362	638	79	5176
21 August	36	43	147	4	3555	563	32	4344
28 August	26	0	6	0	1470	76	8	1560
4 September	36	6	143	5	1917	16	20	2107
11 September	34	7	113	0	1180	6	46	1352
18 September	35	6	42	1	1427	16	84	1576
25 September	35	2	42	0	1234	5	72	1355
2 October	35	2	8	11	1187	2	8	1218
9 October	35	1	6	1	1289	7	28	1332
Totals	691	1330	8444	369	58298	9652	1081	71574

Table V. Weekly Collections of Culex pipiens pallens Adults from 36 Resting Stations

Week beginning	Male No.	Male %	Female No.	Female %	Total No.
29 May	688	54	593	46	1281
5 June	1274	59	1064	41	2338
12 June	2524	57	1892	43	4416
19 June	2015	53	1795	47	3810
26 June	2635	56	2061	44	4696
3 July	1740	47	1977	53	3717
10 July	2680	59	1818	41	4498
17 July	1595	54	1384	46	2979
24 July	2391	58	1759	42	4150
31 July	2428	56	1878	44	4306
7 August	2820	63	1666	37	4486
14 August	2622	60	1740	40	4362
21 August	2265	54	1290	36	3555
28 August	841	58	629	42	1470
4 September	1291	67	626	33	1917
11 September	631	53	549	47	1180
18 September	789	55	638	45	1427
25 September	733	59	501	41	1234
2 October	566	48	621	52	1187
9 October	701	54	588	46	1289
Totals	33229	57	25069	43	58298

Light Trap Collections - Light trap collections were begun on 5 June and extended through 8 October. In planning the survey it was intended to operate four light traps in permanent locations three nights weekly, thus yielding a total of twelve weekly collections. Unfortunately, due to mechanical difficulties, the number of collections for

the entire season totalled only 82 trap nights. Of the four traps in operation, the two which operated most dependably were located on the grounds of the Ueno Park Zoological Gardens in Tokyo, and at a horse stable in an industrial area of Tokyo. The third trap located at a horse stable in a rural area and the fourth trap located in a Japanese residential area operated only sporadically.

Table VI. lists the weekly collections of mosquitoes taken by light traps. As in the case of adult resting station collections, light trap collections showed a distinct peak in the Culex tritaeniorhynchus population during the week of July 24 (Fig. 1). With the exception of Aedes vexans, no other species showed a distinct peak in the population curve; A. vexans showing a small peak during the week of 26 June. The proportion of males taken by light trap was higher than the proportion of males taken from resting station collections (Tables II and VI). Unlike the resting station collections where Culex pipiens was the predominant species taken, light traps yielded 87% Culex tritaeniorhynchus, 7% Aedes vexans, 5% Culex pipiens and 1% miscellaneous species. The discrepancies shown by these two methods of collecting well illustrate the necessity of multiple collection methods for obtaining unbiased data.

Table VI. Weekly Collections of Adult Mosquitoes from Light Traps in 1949

Week beginning	No. trap nights	Anopheles hyrcanus sinensis	Aedes vexans nipponii	Culex pipiens pallens	Culex tritaeniorhynchus	Misc. Species	Totals
5 June	3	0	1	2	6	1	10
12 June	3	0	0	16	2	3	21
19 June	4	0	14	14	14	0	42
26 June	6	0	200	65	90	1	356
3 July	6	1	167	32	189	1	390
10 July	6	2	57	40	681	2	782
17 July	7	4	9	9	366	1	389
24 July	6	24	32	17	1906	4	1983
31 July	5	6	21	10	1737	2	1776
7 August	7	3	14	39	998	5	1059
14 August	6	2	0	43	348	1	394
21 August	4	0	1	3	122	0	126
28 August	3	1	0	6	36	0	43
4 September	1	1	1	8	47	0	57
11 September	3	0	1	8	53	4	66
18 September	5	2	1	21	49	3	76
25 September	3	1	1	27	106	1	136
2 October	4	0	0	8	17	1	26
Totals	82	47	520	368	6767	30	7732
No. males		25	143	249	1463	11	1891
No. females		22	377	119	5304	19	5841
% Females		47	73	32	78	63	75

Animal Bait Trap Collections - Two animal bait traps were operated on the grounds of the Ueno Park Zoological Gardens from 3 July to 15 October 1949. These trap (Fig. 2) are small screened structures of sufficient size to accommodate the larger domestic animals. Two baffles with a ½ inch opening run the length of the sides. The baffles permit mosquitoes to enter the trap, but prevent them from leaving. The traps were placed in the center of the Zoo grounds and were separated by a distance of 65 yards. Two types of collections were made. The first series consisted of operating one trap with a horse as bait three nights weekly. The three horse-trap-night collections were used as an index of weekly mosquito population levels. The second series consisted of alternating animal hosts in the traps to determine host tropisms of the various mosquito species. In all collections, male mosquitoes were only rarely taken, and females were almost invariably found to be engorged.

Figure 2

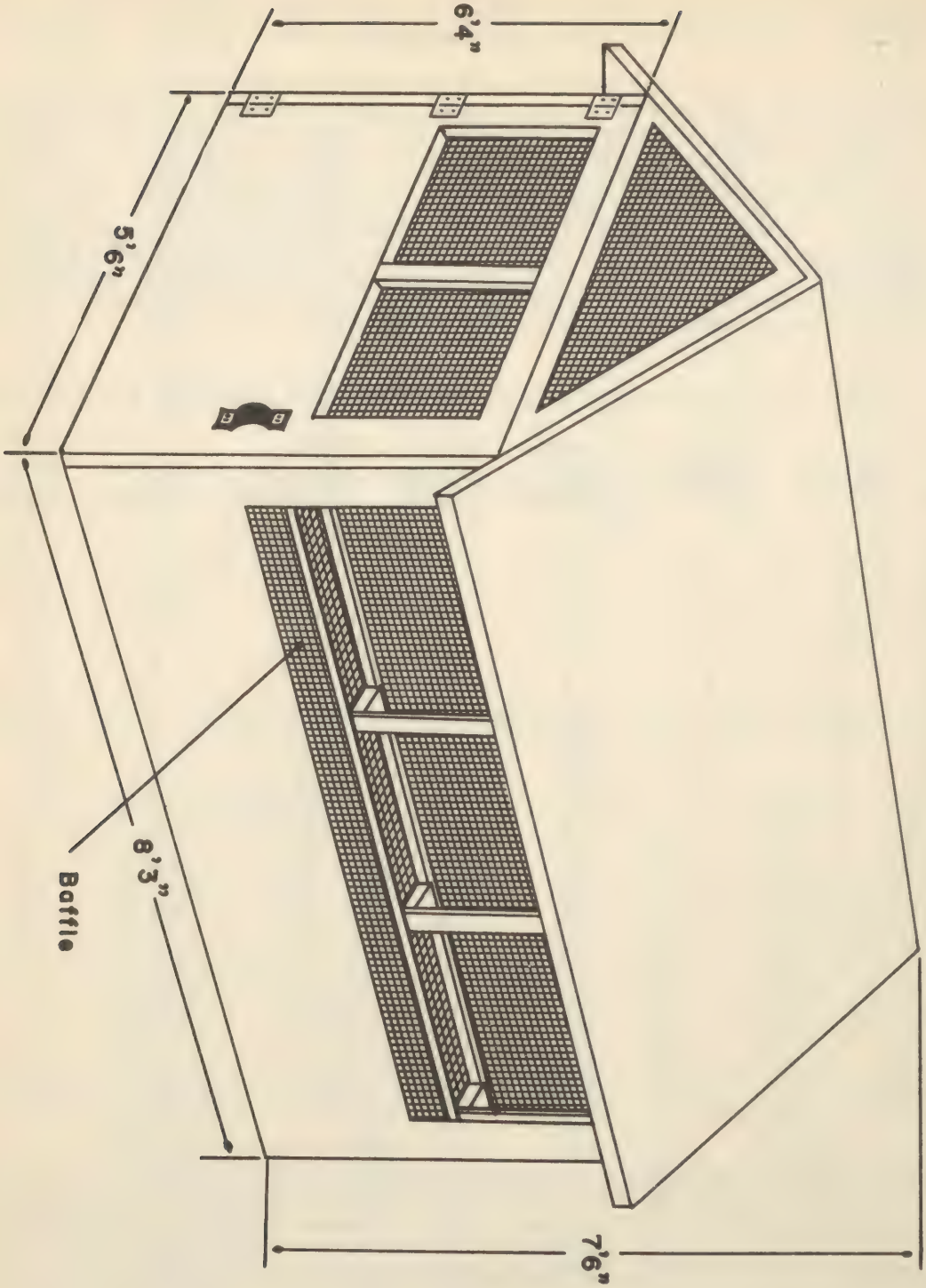

Fig. 2. Animal Bait Trap

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Table VII lists the weekly animal bait trap collections using the three horse-trap-nights as base. As in the case of adult resting station collections, the week of 24 July presented a peak for the Culex tritaeniorhynchus population. This peak showed a sharper spike than the peaks obtained for the species by other collecting methods. In fact, the peak should be considerably larger, since collection of mosquitoes in the animal bait trap during the peak week was incomplete, the collectors being forced by exhaustion to discontinue collection after several hours of fatiguing work. Small peaks were also obtained with Anopheles hyrcanus and Aedes vexans, but with these species the number of mosquitoes taken was relatively small.

Table VII. Weekly Collections of Mosquitoes from Animal Bait Traps

Week beginning	No. horse-trap-nights	Anopheles hyrcanus sinensis	Armigeres subalbatus	Aedes vexans nipponii	Culex pipiens pallens	Culex tritaeniorhynchus	Misc. Species	Totals
3 July	2	16	0	459	18	2746	0	3239
10 July	2	45	1	80	8	1525	2	1661
17 July	3	313	9	102	9	2222	3	2658
24 July	3	323	7	297	3	7750	6	8386
31 July	3	151	9	246	5	3440	9	3860
7 August	3	77	13	31	12	1371	4	1508
14 August	3	57	10	15	18	609	1	710
21 August	3	27	6	26	3	1092	0	1154
28 August	3	52	8	18	1	930	1	1010
4 September	3	62	8	5	3	625	4	707
11 September	3	40	8	19	5	124	2	198
18 September	3	17	7	5	3	83	12	127
25 September	3	43	4	3	7	45	2	104
2 October	3	9	0	3	0	2	0	14
9 October	3	12	1	4	1	1	1	20
Totals	43	1244	91	1313	96	22565	47	25356

Data on host tropisms of mosquito species obtained through the use of animal bait traps are given in Table VIII. When birds were used in the trap, large numbers were used as bait. In the case of domestic animals, only a single animal was used, and for man a Japanese volunteer was employed as bait. Culex tritaeniorhynchus was the predominant mosquito species taken. Birds and fowl did not attract very many mosquitoes, and the number taken are so small that little can be derived from the data. The slight preponderance of C. tritaeniorhynchus in fowl-bait collections is questionable due to the possibility of small residues of mosquitoes being left in the trap following operation of the trap with a horse as bait. In six nights of collection in which man was used as bait, C. tritaeniorhynchus represented 75% of the mosquito catch, while C. pipiens constituted only 16%. The collections with humans are also of interest in that they show larger collections of miscellaneous species, including Aedes togoi, A. niveus nopponicus and A. albopictus, than when domestic animals were used as bait. Data on the mule show that C. tritaeniorhynchus constituted only 35% of the mosquito catch, while 56% of the catch was Aedes vexans. These statistics are based on only one night of trapping and may merely reflect a particularly active flight night for the latter species. Collections from 61 horse-trap-nights yielded 89% C. tritaeniorhynchus and only 5% Aedes vexans. The relationship between the horse and mule is sufficiently close to case doubt on the significance of this discrepancy.

Human Biting Records - Numerous species of mosquitoes occurring in Japan are known to bite man, either as a primary or secondary host. These species include: Anopheles hyrcanus sinensis Wied., Mansonia uniformis (Theob.), Armigeres subalbatus (Coq.), Aedes dorsalis (Meig.), Aedes japonicus (Theo.), Aedes togoi (Theob.), Aedes niveus nipponicus La Casse & Yamaguti, Aedes aegypti (Linn.), Aedes albopictus (Skuse), Aedes

flavopictus Yamada, Aedes vexans nipponii (Theob.) Culex vorax Edw., Culex hayashii Yamada, Culex bitaeniorhynchus karatsuensis Moch., Culex sinensis Theob., Culex whitmorei (Giles)., Culex tritaeniorhynchus Giles, Culex mimeticus Noe, Culex quinquefasciatus Say, and Culex pipiens pallens Coq.

Table VIII. Collections of Mosquito Adults from Animal Bait Traps

Mosquito Species	Man	Horse	Mule	Cow	Sheep	Goat	Pig	Monkey	Chicken	Swallow	Sparrow	Totals
Anopheles hyrcanus	3	1330	1	118	3	2	4	0	0	0	0	1461
A. sineroides	0	0	0	1	0	0	0	0	0	0	0	1
Armigeres subalbatus	0	104	2	26	1	0	5	0	1	0	0	139
Aedes japonicus	0	25	9	1	3	0	0	0	0	0	0	38
A. togoi	5	7	0	1	2	0	0	0	0	0	0	15
A. niveus nipponicus	2	6	0	0	0	0	0	0	0	0	0	8
A. albopictus	3	6	0	2	0	1	2	0	0	0	0	14
A. vexans	1	1356	311	54	139	1	15	0	0	1	0	1878
Culex bitaeniorhynchus	0	16	0	9	0	0	1	0	0	0	0	26
C. tritaeniorhynchus	137	23046	193	2522	438	55	147	0	15	0	0	26553
C. mimeticus	0	0	0	0	0	0	0	0	0	1	0	1
C. orientalis	0	1	0	0	0	0	0	0	0	0	0	1
C. pipiens	30	106	40	11	1	0	11	0	7	2	0	208
Totals	181	26003	556	2745	587	59	185	0	23	4	0	30343
No. Animal-Trap-nights	6	61	1	14	2	2	3	1	4	2	1	97

During 1949, very few day or night biting collections were made. However, data are available from other sources. Petrakis (2) made 1,948 collections totaling 974 man-collecting hours from 27 scattered points in Yokohama during the summer of 1949. All collections were made by Japanese nationals; identifications were made by himself. These collections yielded 3,354 mosquitoes which were taken as follows: Culex pipiens pallens 1,975 (58.9%), Culex tritaeniorhynchus 793 (23.7%), Anopheles hyrcanus sinensis 104 (3.1%), Armigeres subalbatus 27 (0.8%), Aedes vexans nipponii (13, 0.4%), and miscellaneous species 442 (13.1%). Miscellaneous species taken were mostly represented by Aedes albopictus and Aedes togoi. Fig. 3 based on data from Petrakis, shows the seasonal variation in biting rates of Culex pipiens pallens, Culex tritaeniorhynchus and total (all species) during 1949 in Yokohama. The rates illustrated represent the number of mosquitoes biting man per half hour period (number of mosquitoes divided by number of half hour periods). It will be noted that the peak week for all curves in Fig. 3 was the week beginning 24 July. This corresponds with the results obtained by this laboratory using several other methods of collecting in Tokyo.

Night biting collections made by LaCasse (3) and Schenker (4) in Kyoto during the period 1946-1949 are summarized in Table IX for rural areas in that city and in Table X for urban areas. These data are based on one rural area collecting station and two urban area collecting stations. The figures given represent number of mosquitoes taken, not biting rates. A comparison of the two tables shows considerably larger collections from the rural area, despite the fact that a greater number of collecting hours was devoted to the urban stations. All species were found to bite man more frequently in rural areas. In contrast to the biting data from Yokohama, where Culex pipiens was taken more frequently than Culex tritaeniorhynchus, the data from urban areas in Kyoto show that C. tritaeniorhynchus was the more frequent biting species.

Figure 3.

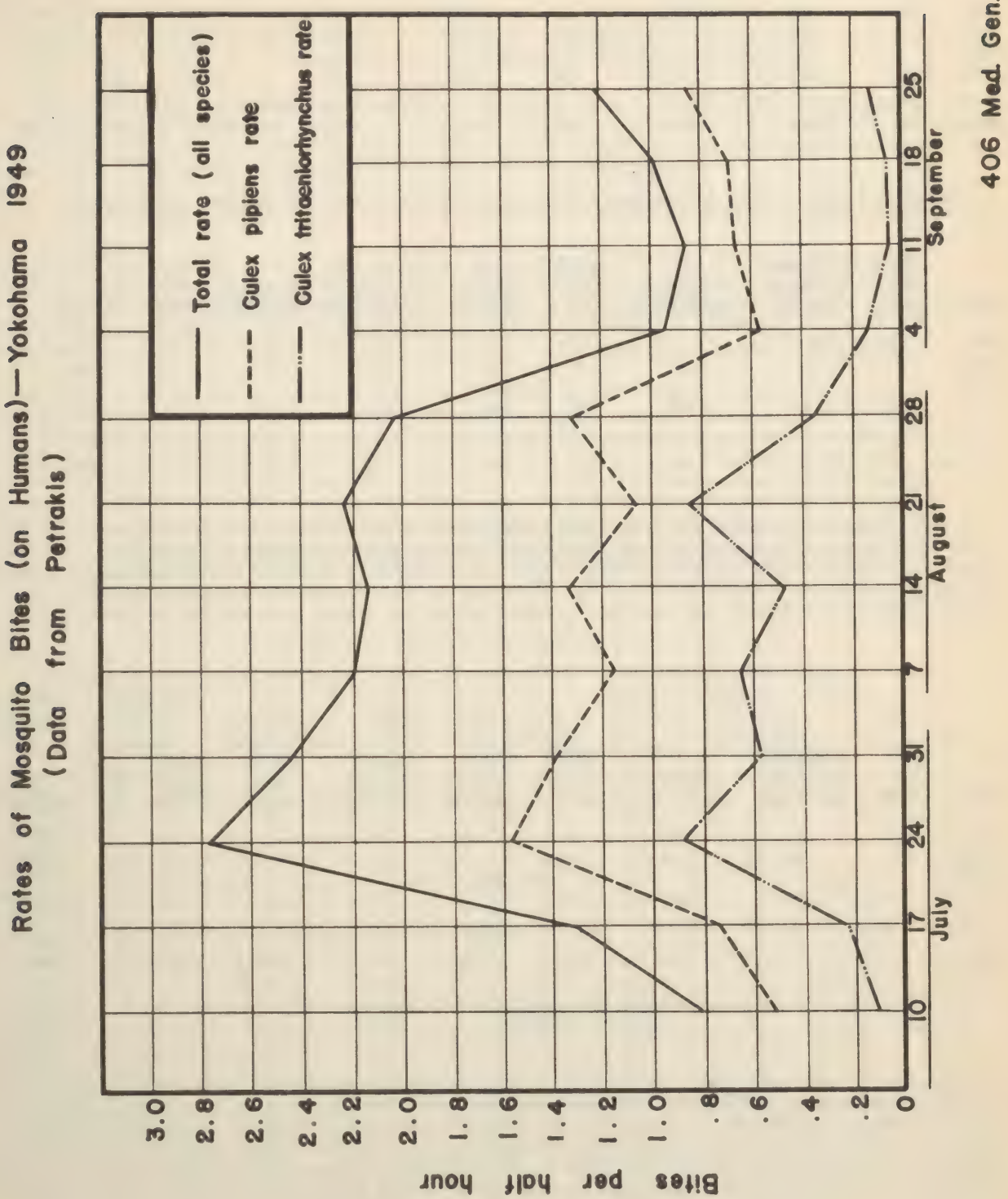
Rates of Mosquito Bites (on Humans)—Yokohama 1949
(Data from Petrakis)
Total rate (all species)
Culex pipiens rate
Culex tritaeniorhynchus rate
Bites per half hour
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
10
17
24
31
July
7
14
21
28
August
4
11
18
25
September
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Table IX. Night Biting Collections - Rural Area, Kyoto (Data from LaCasse and Shenker)

Year	No. Man Hours	Anopheles hyrcanus sinensis	Armigeres subalbatus	Aedes vexans nip.	Culex pipiens pallens	Culex tritaen-iorhynchus	Mansonia uniformis	Misc. spp.	Totals
1946	27	168	19	0	45	400	1088	45	1765
1947	33	159	10	360	128	328	348	159	1500
1948				No data					
1949	36	123	0	62	288	54	101	25	653
Totals	96	450	29	430	461	782	1537	229	3918

Table X. Night Biting Collections - Urban Area, Kyoto (Data from LaCasse and Shenker)

Year	No. Man Hours	Anopheles hyrcanus sinensis	Armigeres subalbatus	Aedes vexans nip.	Culex pipiens pallens	Culex tritaen-iorhynchus	Mansonia uniformis	Misc. spp.	Totals
1946	39	55	26	0	34	498	2	13	628
1947	27	24	21	5	187	152	0	72	461
1948				No data					
1949	72	119	39	96	179	100	22	75	630
Totals	138	198	86	101	400	750	24	160	1719

LaCasse (3) and Shenker (4) also provide some data on day biting collections (see Table XI). Aedes albopictus, Aedes flavopictus, Aedes vexans and Armigeres subalbatus are shown to be the most important day biting species in the vicinity of Kyoto. Experience in Tokyo, however, has shown that with the exception of parks, cemetery grounds, bamboo groves (very few in Tokyo) and some rural areas, little day biting activity can be noted.

Table XI. Day Biting Collections - Bamboo Grove, Yodo (Data from LaCasse and Schenker)

Year	No. Man Hours	Anopheles hyrcanus sinensis	Armigeres subalbatus	Aedes vexans nip.	Culex pipiens pallens	Culex tritaen-iorhynchus	Aedes albo-pictus	Aedes flavo-pictus	Misc. Spp.	Totals
1946	14	7	224	0	29	27	939*		6	1232
1947	8	2	57	101	0	2	66	581	0	809
1948				No data						
1949	7.3	0	108	12	4	0	330	185	0	639
Totals	29.3	9	389	113	33	29	369/	766/	6	2680

* During 1946 Aedes albopictus and Aedes flavopictus were not recognized as separate species.

Larval Collections - Larval collecting areas throughout the city of Tokyo were established by designating a one block radius around each adult resting station as a larval collecting area, to be checked once weekly. A few additional permanent larval stations were set up in some locations such as the moat surrounding the Imperial Palace, and in rice paddy areas. Larval collections were made in the conventional manner, with breeding intensities being indicated by "number of larvae per dip", and with notation of the estimated size of the breeding area. Early in the survey it became apparent that this method

of collecting, while satisfactory for indicating species location or species habitat breeding, was not an entirely satisfactory yardstick for the comparison of breeding levels of different species. In the assessment of larval populations, there are three factors to be considered, namely, number of collections, density of larval breeding, and the size of breeding areas. If larval collections for each species are categorized under a series of classifications of the three factors, the multiplicity of categories becomes so unwieldy that the whole body of data becomes unintelligible. With a total of 1,338 larval collections made during the course of the season, it was found entirely impractical to attempt to measure population changes in the above manner.

Recognizing this difficulty, an attempt was made to develop a larval breeding index by assessing numerical values to breeding density as measured by "number of larvae per dip" and to area size, and multiplying these factors by the number of collections. This index might be expressed by the following formula:

Larval breeding index = No. larval collections x breeding density x breeding area.

After a few attempts it became apparent that this larval breeding index was too inaccurate to be of any value. Breeding areas are usually very difficult to measure, since breeding is rarely uniform through a body of water. There is also much room for disparity in the evaluation of density of breeding, particularly where breeding is heavy. For these reasons, data herein presented are limited to the number of larval collections.

A summary of weekly larval collections is given in Table XII. Based on number of collections, the peak larval breeding for Culex pipiens occurred during the week of 19 June, one week prior to the peak week for adults of this species. The larval peak for Culex tritaeniorhynchus occurred during the week of 24 July, which was also the peak week for adults of this species. Taking all or total species, the peak of larval breeding occurred during the week of 10 July, one of the two peak weeks for total mosquito adults. Larval collections of Culex pipiens showed a rapid and distinct decline following the peak week, whereas the population curve for the adults of this species showed a high population level for seven weeks before the decline set in. The reason for this discrepancy is not clear.

Table XII. Weekly Larval Collections

Week beginning	Anopheles hyrcanus sinensis	Armigeres subalbatus	Aedes togoi	Aedes albopictus	Culex pipiens pallens	Culex tritaeniorhynchus	Misc. Spp.	Totals
29 May	0	0	5	2	18	1	1	27
5 June	0	1	8	5	34	0	2	50
12 June	1	2	13	8	42	1	6	73
19 June	1	3	34	6	60	2	2	108
26 June	3	3	38	11	46	9	11	121
3 July	0	4	24	10	40	3	10	91
10 July	6	3	37	19	42	7	16	130
17 July	10	3	20	13	34	7	12	99
24 July	4	3	17	11	23	15	6	79
31 July	8	2	21	9	26	8	14	88
7 August	5	5	11	6	23	13	8	71
14 August	4	4	11	5	20	11	12	67
21 August	0	7	6	9	23	3	8	56
28 August	0	3	10	6	12	3	4	38
4 September	1	3	11	8	12	5	7	47
11 September	1	2	11	9	6	2	3	34
18 September	1	3	11	8	14	0	10	47
25 September	1	3	8	11	15	5	3	46
2 October	0	2	7	6	17	2	5	39
9 October	0	2	4	4	14	0	3	27
Totals	46	58	307	166	521	97	143	1338

Table XIII provides a breakdown of larval collections by breeding habitat. Difficulties in the classification of a number of breeding habitats have resulted in the distortion of some data. Care must also be used in the interpretation of these data in other respects. For example, out of 521 collections of Culex pipiens, 114 were taken in artificial containers, while only 55 were taken in ditches. From this it might be concluded that C. pipiens is predominantly an artificial container breeder. Actually, larval densities were usually far greater in ditches, and the breeding area involved was considerably greater in ditches. The number of collections from artificial containers is high in general due to frequent collections from Japanese cemeteries, where small numbers of mosquito larvae are almost invariably found in small tanks, flower urns, bamboo pots, ets. It is of interest to note that Aedes togoi was taken commonly in the larval stage, but adults were taken only infrequently. Similarly, Aedes albopictus was taken commonly in the larval stage, but adults were taken only during day biting collections and rarely from resting stations and animal bait traps. Culex tritaeniorhynchus ranked only fourth in the frequency of larval collection.

As stated previously, larval collection areas were almost invariably associated with adult resting stations, half of which were located in urban areas, the remaining half being in rural areas. A comparison of the number of larval collections of C. pipiens and C. tritaeniorhynchus from urban and rural areas can therefore be made. Culex pipiens collections totalled 521 of which 223 or 43% were from urban areas, while 298 or 59% were from rural areas. This conflicts with the data obtained by adult resting stations collections (Table III) where this species showed a slight predominance in urban areas. Collections of C. tritaeniorhynchus larvae totalled 97, of which 32 or 33% were taken in urban areas, while 65 or 66% were taken in rural areas. The predominance of this species in rural areas was more accentuated in the case of adults.

Weather Factors Affecting Mosquito Populations - Weather factors should be of considerable importance in determining mosquito population levels as is amply demonstrated by a review of the literature. Unfortunately, the many variables are multiplied by the number of mosquito species considered here and as a result generalizations on the subject are exceedingly dangerous. In the present study some of the expected relationships between weather and population trends did not materialize. Correlation between adult population levels and four weather factors was sought. These factors were temperature, relative humidity, saturation deficiency* and precipitation. Most authorities consider saturation deficiency to be a better index of humidity effects than relative humidity since it expresses temperature variants as well as humidity variants. Trends in larval populations were studied in relation to precipitation and temperature.

Figure 4. illustrates the relationships of the population curve for all mosquitoes taken from adult resting stations to four weather factors expressed as mean weekly temperature, mean weekly relative humidity, mean weekly saturation deficiency and total weekly precipitation. Total mosquito population rose rapidly from 29 May to the week of 12 June. This period saw a rise in temperature and relative humidity expressing itself as a drop in the saturation deficiency. A sharp but irregular increase in population followed in the ensuing four weeks which were characterized by a fairly stable relative humidity, a sharp rise in temperature during the latter half of the period, and a gradual rise in saturation deficiency. Twin peaks occurred two weeks apart during the weeks of 10 July and 24 July. The intervening week saw an abrupt rise to peak level in saturation deficiency, thus reflecting a sharp rise in temperature (the peak for the season) and a corresponding sharp drop in relative humidity. Following the population peak reached during the week of 24 July, a sharp decline in the adult population occurred during the next five weeks, terminating the week of 28 August when a severe typhoon struck the Tokyo area. The population remained fairly stable at a low level thenceforth, until the survey was terminated on 15 October. During the

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* Saturation deficiency is defined as the difference between the saturation vapor pressure at a given temperature and the vapor pressure at a given relative humidity and temperature.

Sat. defic. = sat. vap. press. - (sat. vap. pressure x rel. humid.)

Table XIII. (Part 1) Larval Collections by Breeding Place

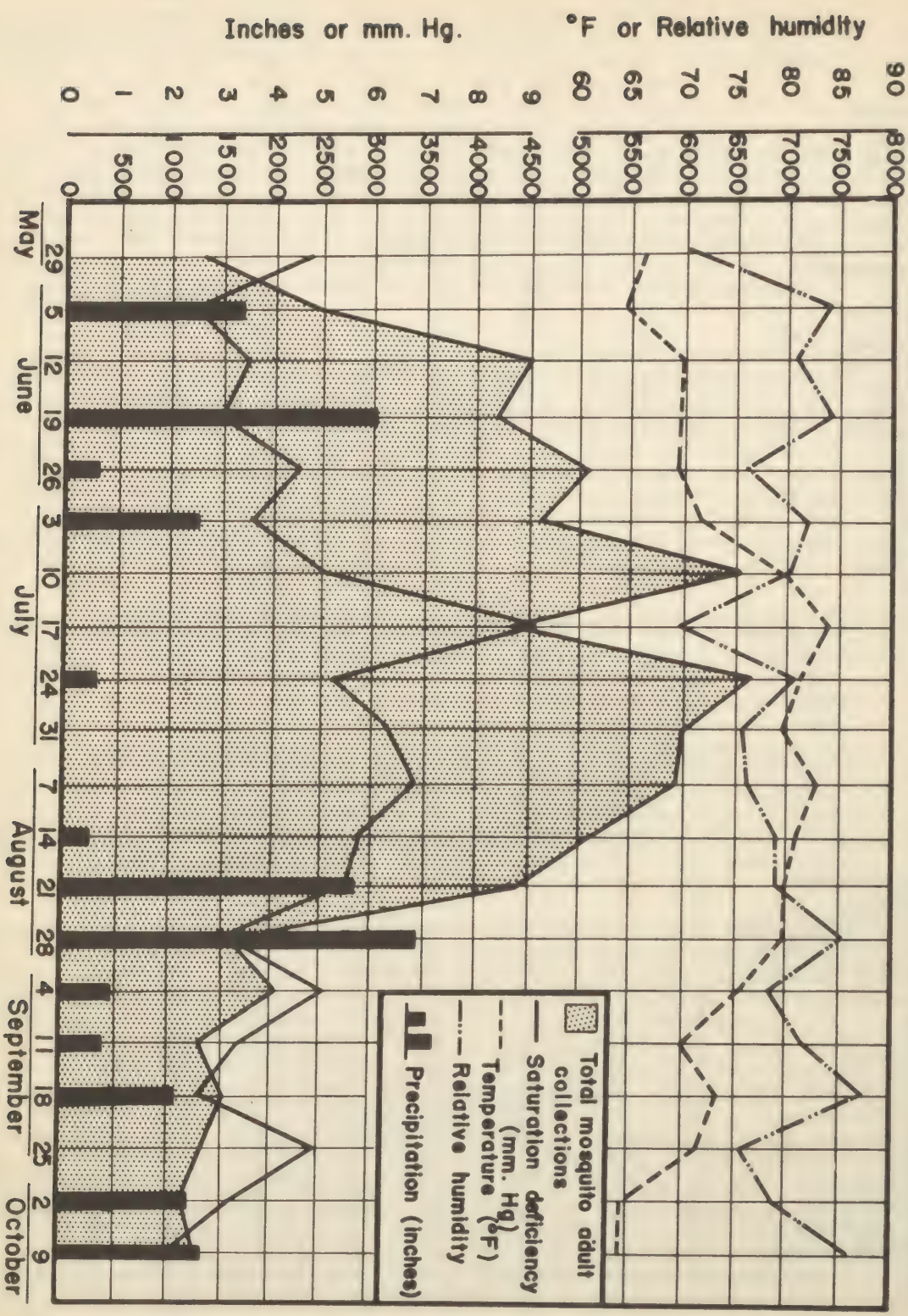
Species	Artificial Container	Nightsoil Container	Benjo	Tank	Sump	Ground Pool	Bamboo Stump	Ditch	Stream
Anopheles hyrcanus	7	5	0	7	1	1	0	6	1
A. sineroides	1	0	0	0	0	0	0	0	0
Uranotaenia bimaculata	1	8	3	0	0	0	0	0	0
Armigeres subalbatus	10	36	9	1	0	0	0	1	0
Tripteroides bambusa	0	1	0	0	0	0	0	0	0
Aedes japonicus	10	2	5	5	0	0	1	0	0
A. togoi	152	29	23	57	5	3	0	1	0
A. niveus nip.	7	0	0	1	0	0	0	0	0
A. albopictus	84	16	19	5	0	1	3	2	0
A. flavopictus	0	0	0	0	0	0	0	0	0
Culex vorax	16	12	4	5	3	1	0	0	0
C. hayashii	1	1	0	0	0	1	0	1	1
C. tritaeniorhynchus	11	9	0	19	2	8	0	8	1
C. mimeticus	1	0	0	0	0	0	0	2	0
C. pipiens	114	67	19	49	75	75	0	55	7
Mansonia ochracea	0	0	0	0	0	0	0	0	0
Undetermined Species	0	0	0	0	0	0	0	0	0
Totals	415	186	82	149	86	90	4	75	10

Table XIII. (Part 2) Larval Collections by Breeding Place

Species	Pond	Rice Paddy	Marsh	Sewer	Rock Pool	Tomb-stone	Well	Lake	Total
Anopheles hyrcanus	6	11	1	0	0	0	0	0	46
A. sineroides	0	0	0	0	0	0	0	0	1
Uranotaenia bimaculata	0	1	0	0	0	0	0	0	13
Armigeres subalbatus	0	1	0	0	0	0	0	0	58
Tripteroides bambusa	0	0	0	0	0	0	0	0	1
Aedes japonicus	0	0	0	0	1	14	0	0	38
A. togoi	8	0	0	0	5	23	1	0	307
A. niveus nip.	0	0	0	0	0	6	0	0	14
A. albopictus	8	0	0	0	11	17	0	0	166
A. flavopictus	0	0	0	0	0	1	0	0	1
Culex vorax	0	1	0	0	1	0	1	0	44
C. hayashii	14	0	0	0	0	0	3	2	24
C. tritaeniorhynchus	14	13	9	0	1	0	2	0	97
C. mimeticus	0	0	0	0	0	0	0	0	3
C. pipiens	12	23	6	1	2	4	12	0	521
Mansonia ochracea	0	0	2	0	0	0	0	0	2
Undetermined Species	2	0	0	0	0	0	0	0	2
Total	64	50	18	1	21	65	19	2	1338

steady decline of the five week period beginning 31 July, the temperature remained high and there was a gradual increase in relative humidity. The apparent significant factor in the decline was the drought extending from the week of 10 July through the week of

Figure 4



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14 August.

The relationship between weather and the Culex pipiens adult population based on resting station collextions is illustrated in Figure 5. Since the population curve for this species resembles the curve for the entire mosquito population, many of the same relationships to weather factors pertain. The early population build-up paralleled a rise in temperature and relative humidity, but unlike the curve for the entire mosquito population was followed by two small peaks and then a high level plateau, giving the entire population curve a more generalized plateau appearance. As in the case of the total mosquito population, a decline occurred during the week of 17 July, which was characterized by a marked rise of saturation deficiency to peak level. Culex pipiens maintained a high population level from 12 June through the week beginning 21 August. This period showed a high mean temperature level, but apparently the six week drought effected a reduction in population at the end of the period.

Figure 6 illustrates the relationship between the four weather factors and the Culex tritaeniorhynchus population measured by adult resting station collections and by animal bait trap collections. The curve for the species obtained by adult resting station collections remained at a low level through the week of 26 June, rose rapidly through the week of 10 July, paralleling the sharp rise in temperature. As in the case of Culex pipiens and the mosquito population as a whole, the week of 17 July in which the saturation deficiency rose to peak level saw a decrease in the C. tritaeniorhynchus population. The following week the temperature and relative humidity were high but neither reached peak levels. Rainfall during this week totalled 0.64 inches, but no rain fell during the preceding two weeks. Following the peak week the population declined steadily until by the week of 28 August collections were negligible. This decline again can only be attributed to the drought occurring during the period 10 July - 20 August, since temperature and relative humidity remained high during the period of decline. During the week of 28 August, which ushered in the low population level, Tokyo was struck by a severe typhoon.

The population curve for C. tritaeniorhynchus based upon animal bait trap collections is markedly dissimilar from that obtained for the species by adult resting staion collections. This disparity may be attributed to the possibility of animal bait traps reflecting variations in mosquito biting activity as well as variations in mosquito population levels. Collections from animal bait traps commencing with the week of 3 July, showed a high population level initially and then showed a marked decrease in yield the following week. This decrease is at odds with the population increase demonstrated by adult resting station collections and by light trap collections. The week in which it occurred, 10 July, saw the sharpest climb in temperature for the summer, though not the peak temperature, and a slight drop in relative humidity. Contrary to the decrease shown in the C. tritaeniorhynchus population during the week of 17 July by resting station and light trap collections, animal bait trap collections showed distinct increase in yield, despite peak saturation deficiency for the summer. The following week, 24 to 30 July, presented a huge rise in the number of C. tritaeniorhynchus taken by animal bait trap. While this week was also the peak week for the species by all methods of collection, (Fig. 1), the accentuated rise in animal bait trap collections is of added significance since it indicated a decided increase in the biting intensity of the species. Looking for an explanation in the weather, one finds a slight drop from the peak temperature for the summer, and a rise in relative humidity, both being reflected by a distinct drop in the saturation deficiency. The extent to which these weather changes influenced the biting rate of the species during this week remains questionable. During the week of 31 July a very marked drop in animal bait trap catches took place, with the explanation of this phenomenon again apparently limited to the effects of the summer drought. The decline continued at a steady rate until the week of 11 September when animal bait trap yields were reduced to a minimum.

Figure 7 illustrates the relationship between larval curves based on the number of weekly collections, mean weekly temperature and total weekly precipitation. The curve for total larvae of all species shows a sharp rise from 29 May to the week of 26 June during a period of slowly rising temperatures and light to heavy rainfall. An unexplained drop occurred during the week of 3 July, followed by a rise to peak level during the week of 10 July. During the ensuing period of little

Figure 5

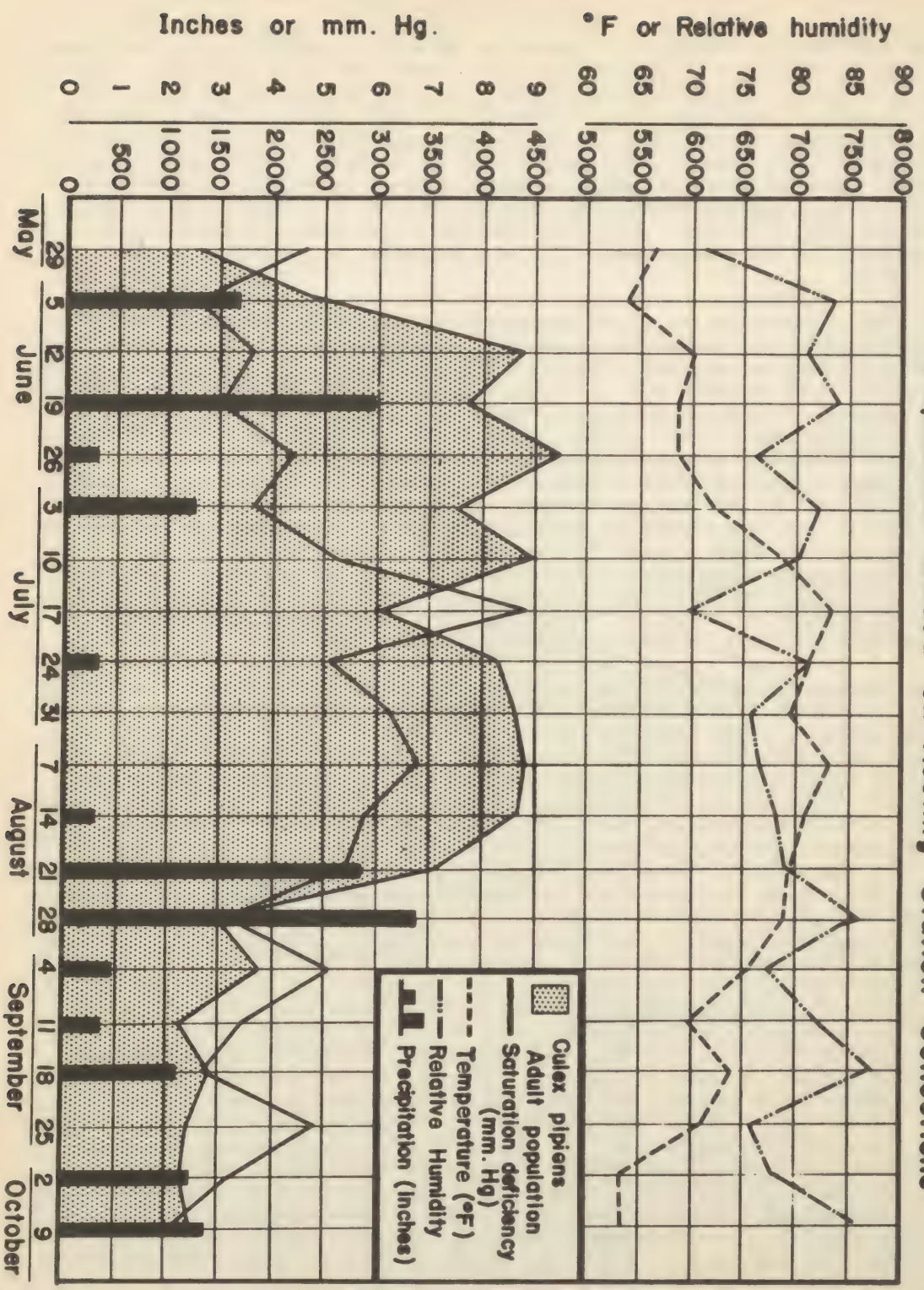

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Figure 6

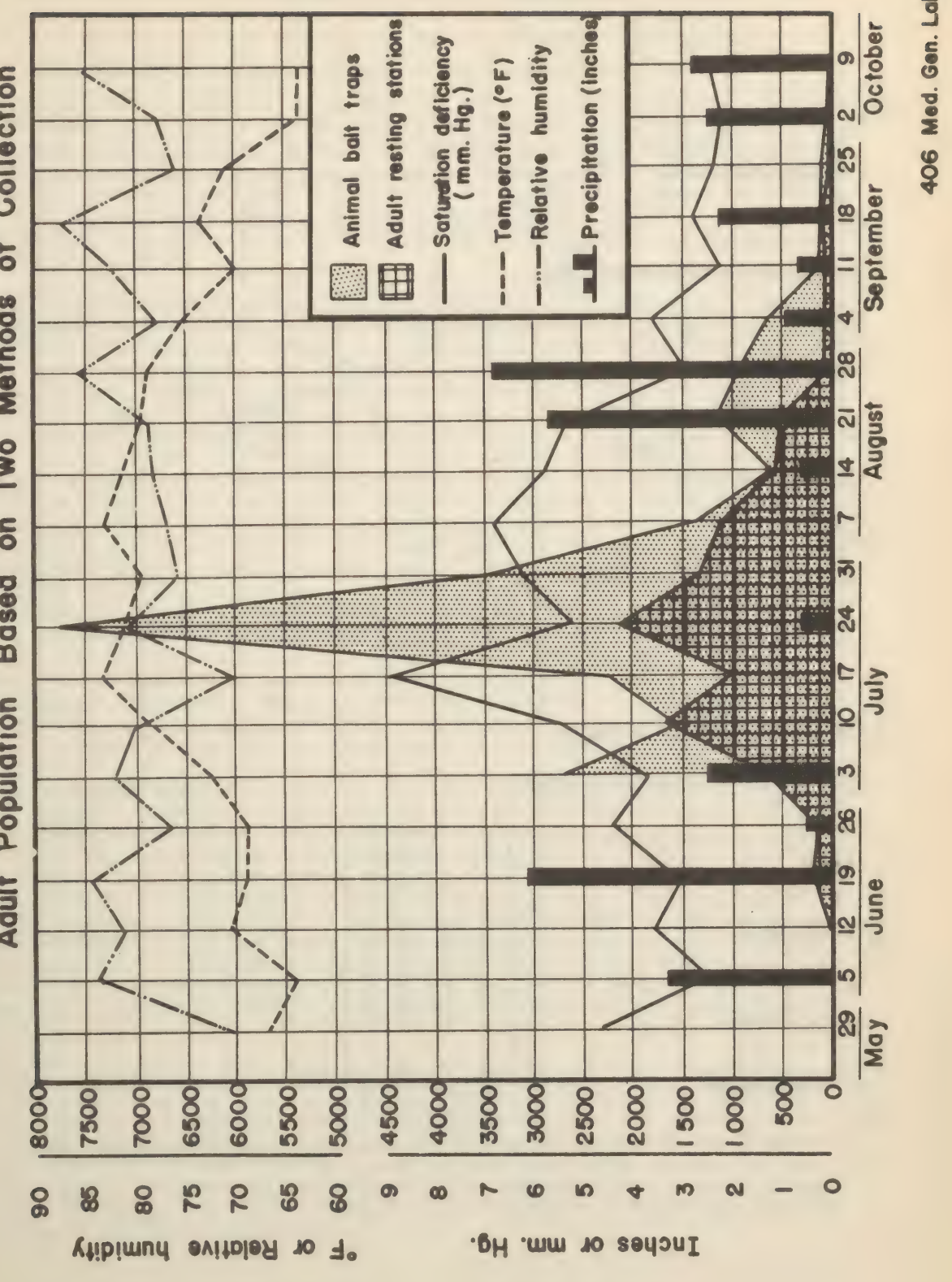

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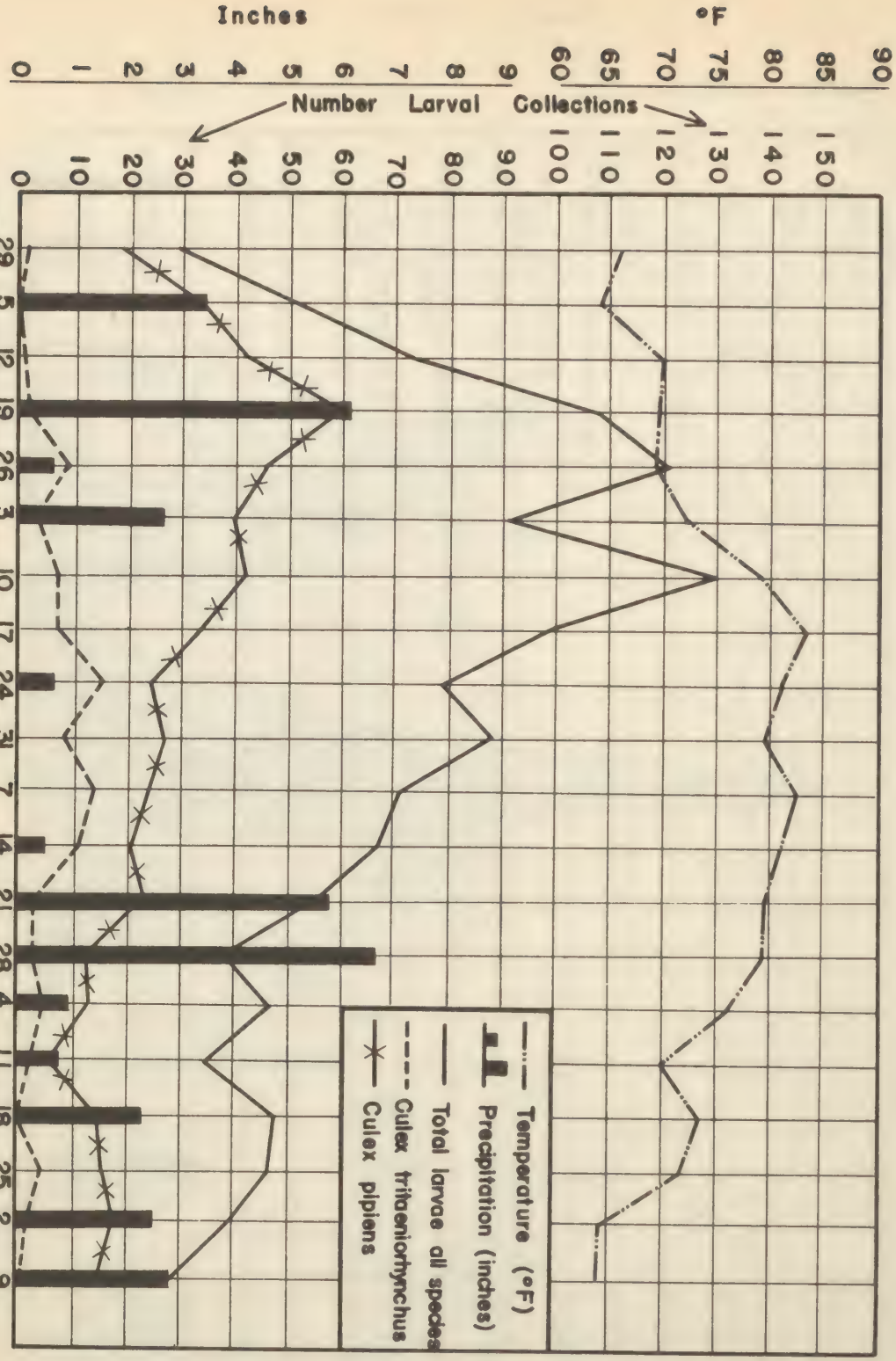
Figure 7



Relation of Weather Factors to Larval Mosquito Breeding

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rainfall there was a sharp drop in larval collections, until the week of 28 August, when the population levelled off following the onset of heavy rains. During the period of larval decline, the temperature remained high. The curve for Culex pipiens larvae follows that of the total larval curve fairly closely, and in general the same observations pertain. Culex tritaeniorhynchus showed no marked increases or decreases but remained at a low stable level during much of the season. The peak for this species took place during the week of 24 July, the peak week for the adults of this species.

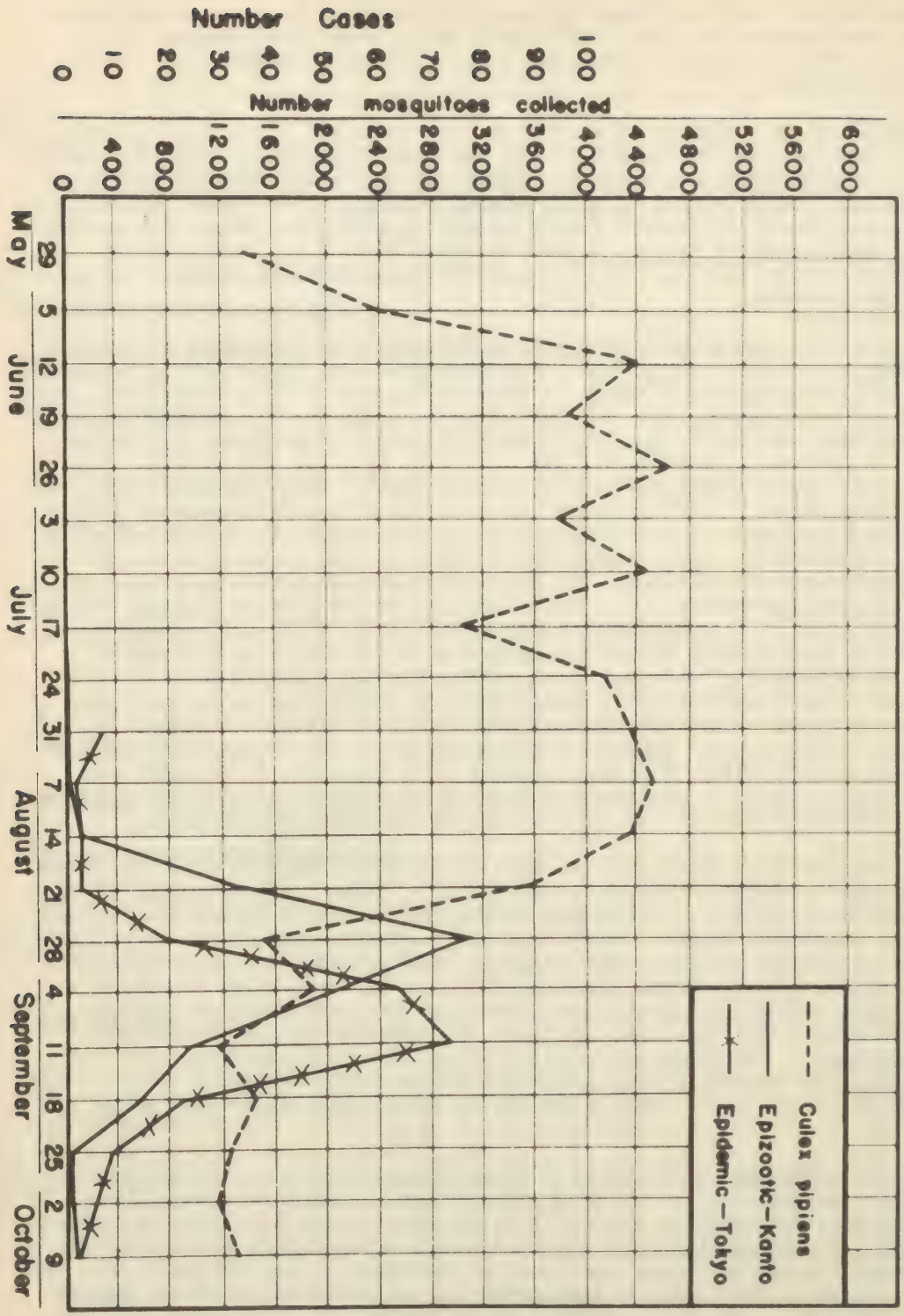
An analysis of the relation of weather factors to mosquito populations leads to the conclusion that any of several factors may directly influence a population, but a weather factor influences mosquito populations only within certain intrinsic limits and within limits imposed by other weather factors. Thus, if a correlation is sought between weekly population trends throughout a breeding season and weekly changes in a single weather factor, no such correlation will be found to apply. However, marked changes in any given factor almost invariably are reflected in mosquito population levels.

Relation of Mosquito Population Trends to Epizootics and Epidemics of Japanese B Encephalitis - An attempt to correlate mosquito population trends with epizootics and epidémics of encephalitis in Japan is beset with a number of difficulties. During the past twenty years considerable information on mosquito populations and mosquito biologies has been gathered by Japanese entomologists and virologists, particularly in relation to Japanese B Encephalitis (5), (6), (7), (8), (9), (10), (11). Most of these data are based on sporadic collections of limited scope, usually from animal shelters or from houses. Little attempt has been made to carefully measure population trends on a randomized basis using samples or collections of statistically significant size and checking these trends by multiple sampling methods. Since the war ended, work undertaken by Americans, has been mainly pointed toward securing taxonomic and biological information (3), (14) and toward the collection of large volumes of mosquito material for virus isolation tests (12), (13), (14), (15). Reports on the incidence of encephalitis in man and animals also are liable to fallibility. Prior to 1948, reporting of encephalitis in horses was not required under law and diagnosis was not entirely reliable. Similarly, the information on the incidence of encephalitis in man is not entirely reliable due to problems of diagnosis and, in the past, lack of reporting. Japanese B Encephalitis was not a reportable disease on a national basis until 1946. For these reasons it is hazardous to accredit some, though certainly not all, of the accepted conclusions based on earlier work relative to the role mosquitoes play in the epidemiology of Japanese B Encephalitis.

During the year 1949 an attempt was made to correlate mosquito population trends with the epidemic in Tokyo. The equine population of Tokyo is rather small and as a result epizootics are not of sufficient size or definition to attempt correlation with mosquito population trends. For this reason correlation has been sought with the epizootic in the Kanto area, which included Tokyo and its six neighboring prefectures, Ibaraki, Tochigi, Gumma, Saitama, Chiba and Kanagawa. Epidemic data presented are based on "date of onset", but since this information was not available for the Kanto epizootic it has been necessary to construct the epizootic curve on "date of reporting". All cases included in the epidemic data are clinically, but not necessarily, serologically confirmed cases. Data on the incidence in horses are based mainly on suspect cases, only a few having been clinically or serologically confirmed.

Figure 8 illustrates the relation of Culex pipiens population levels, based on resting station collections, to the 1949 epidemic and epizootic of encephalitis. It will be noted that this species shows no single distinct peak but rather an irregular plateau curve. The population level remained near the peak level at the onset of the Kanto epizootic and also at a high level at the onset of the Tokyo epidemic. The peak of the epizootic came only one week after the sharp decline in the C. pipiens population, while the peak of the epidemic which occurred on 12 September followed the population decline by three weeks.

Figure 8



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The relation of Culex tritaeniorhynchus population curves to the 1949 epizootic and epidemic are illustrated in Figure 9. Population levels are indicated by two methods of collection, adult resting station collections and animal bait trap collections, both of which indicate distinct peaks during the week of 24 July. This peak was also confirmed by light trap collections. The unusual nature of the perpendicular rise and fall in the animal bait trap collections of Culex tritaeniorhynchus is illustrated by the collections made during that period.

20 July	415	
23 July	788	
25 July	4329	(not all adults taken from trap)
27 July	2706	
30 July	715	

This information places the peak of the population on 25 or 26 July 1949.

An interval of five weeks occurred between the peak of the C. tritaeniorhynchus population and the peak of the epizootic. The interval between the peak of the epidemic and the peak C. tritaeniorhynchus population was seven weeks. Two weeks separated the peaks of the epizootic and the epidemic. As previously stated, the curve for C. tritaeniorhynchus obtained by resting station collections is a more direct estimate of population levels, while that obtained by animal bait traps probably reflects activity of the species as well as population levels. As such the curve obtained by animal bait traps may be of added significance in relation to epizootics and epidemics.

Examination of Tables IV and VII show that Anopheles hyrcanus reached peak level during the week 24 July and the relationships illustrated in Figure 9 for Culex tritaeniorhynchus also apply to this species. In the case of Aedes vexans, resting station collections showed a peak during the week of 10 July, while animal bait trap collections indicated a primary peak during the week of 3 July and a secondary peak during the week of 24 July. Whether this discrepancy is due to the small size of samples involved, or to the importance of mosquito activity being an added measure of animal bait trap collections, cannot be determined. Aedes vexans larvae were not found at any time during the 1949 season, and the peak weeks indicated by animal bait traps may reflect heavy flight nights for this species. This species is known to be a strong flier in the United States.

Using mosquito collection data from Kitaoka, Miura, et al (5), a similar comparison has been attempted between the encephalitis epizootic and epidemic of 1948 and the Culex tritaeniorhynchus population of that year. As in 1949, epizootic data are based on date of reporting for the Kanto areas, while epidemic data are based on date of onset of cases in Tokyo. Equine cases reported are almost entirely suspect cases, while incidence in humans is based on clinically confirmed and in a number of instances serologically confirmed cases. Figure 10 illustrates the relations between the 1948 epizootic and epidemic and the Culex tritaeniorhynchus population. During 1948, the apparent peak of the C. tritaeniorhynchus population was the period 17-19 July, based on limited collections from a horse stable, a pig farm, and a goat farm near Tokyo (5). The peak of the epizootic was reached during the week of 25 July, while the peak of the epidemic occurred on 7 August. Thus in the two year period 1948-1949, it appears that the interval between the peak C. tritaeniorhynchus population and the peak of the epizootic varies from 1-5 weeks, while the interval between the peak population for this species and the peak of the epidemic varies from 3-7 weeks. It is of interest to note that in both years a two week interval separated the peak of the epizootic and the peak of the epidemic, the epizootic occurring first in both years.

Discussion - The literature pertaining to mosquito transmission of Japanese B Encephalitis has grown considerably in the last fifteen years. Conclusions on the importance of various mosquito species in the transmission of the disease appear to have been frequently made based on limited information and occasionally with a disregard for the complexity of factors involved in evaluating the importance of mosquito species as vectors or potential vectors. A partial list of these factors would include the biting habits, host tropisms, flight range, seasonal prevalence, and the occurrence of species strains of mosquitoes. Physiological factors

have been demonstrated to be important in determining whether or not a species is an efficient or inefficient vector of a disease, as in the case of malaria where the endemicity may either be occasioned by a "poor" vector species occurring in great numbers or a "good" vector species with a small population serving as the threshold level of transmission.

Much significant information has been obtained through experimental work on the ability of mosquito species to transmit Japanese B Encephalitis virus to mice (6), (7), (16), (17), (18), (19), (20), (21), and by the isolation of Japanese B Encephalitis virus from wild caught mosquitoes in nature (8), (9), (10), (15), (22), (26). Table XIV provides a brief summary of information now available on the relative importance of a number of mosquito species as possible vectors of this disease. This table is based both on American and Japanese reports. Information on the human attack rate is variable due to localized breeding and limited flight range of some species, and thus the biting intensity will vary considerably with locality. The human attack rate of various mosquito species has received only limited attention and further investigation in this field is highly desirable. Additional data on the population levels of mosquito species prior to epidemics are still needed. As mentioned earlier, much of this type of information gathered previously is subject to some criticism, such as inadequate sampling. Collections of mosquitoes for virus isolation attempts have heretofore been taken mainly from animal shelters with the result that the predominant species collected has been Culex tritaeniorhynchus. The failure to isolate virus from wild caught mosquitoes of the several species listed in Table XIV may merely reflect the heavily weighed type of collecting.

The results obtained in 1949 from a correlation of mosquito population curves with the epizootic and epidemic of Japanese B Encephalitis have been interesting in that they show a relationship considerably at variance with some conclusions present in the literature. Yamada, on the basis of a ten year study of Japanese B Encephalitis in Okayama, has been quoted as maintaining that the peak of the Culex tritaeniorhynchus population always preceded the peak of an epidemic by about two weeks (18). During 1949, investigation by this laboratory found a seven week interval between these peaks. These findings were confirmed by the independent investigations of independent Japanese workers (23). While the very nature of the abrupt perpendicular rise and rapid decline of Culex tritaeniorhynchus populations suggests a correlation with epidemics and epizootics of this disease, the interval between the C. tritaeniorhynchus peak and the peak of epidemics and epizootics appears to vary considerably and as a result Yamada's pre-epidemic peak period can no longer be considered as unvarying.

LaCasse (3) has maintained that the abruptness of the perpendicular rise and fall of Culex tritaeniorhynchus populations reported by various Japanese workers has been exaggerated due to the methods of collection they employed. As previously indicated, we have demonstrated two types of curves for the population of this species in Tokyo during 1949. By collection from adult resting stations and from light traps we have obtained something approximating a normal distribution curve. However, a distinct spiked curve appeared when data from animal bait traps were utilized. These discrepancies obtained by the various methods of collection are interpreted to mean that adult resting stations are a close index of population levels while animal bait traps provide data reflecting biting activity as well as population levels. It is also true that light traps should serve as an index of flight activity as well as population levels, but the operation of light traps was disorganized and interrupted by mechanical failure. The yield was small when compared with the yields from adult resting station and animal bait trap collections, so that the data obtained from light traps may be of questionable statistical significance. Adult resting station collections totalled 71,574; animal bait trap collections totalled 25,356 and light traps yielded 7,732 mosquitoes. It is of interest to note that when animal shelters serving as adult resting stations are segregated from the remaining adult resting stations, the collections of Culex tritaeniorhynchus from the former provide a curve with a peak intermediate between the peak of the curve obtained from animal bait traps and the peak of the curve from all adult resting station collections.

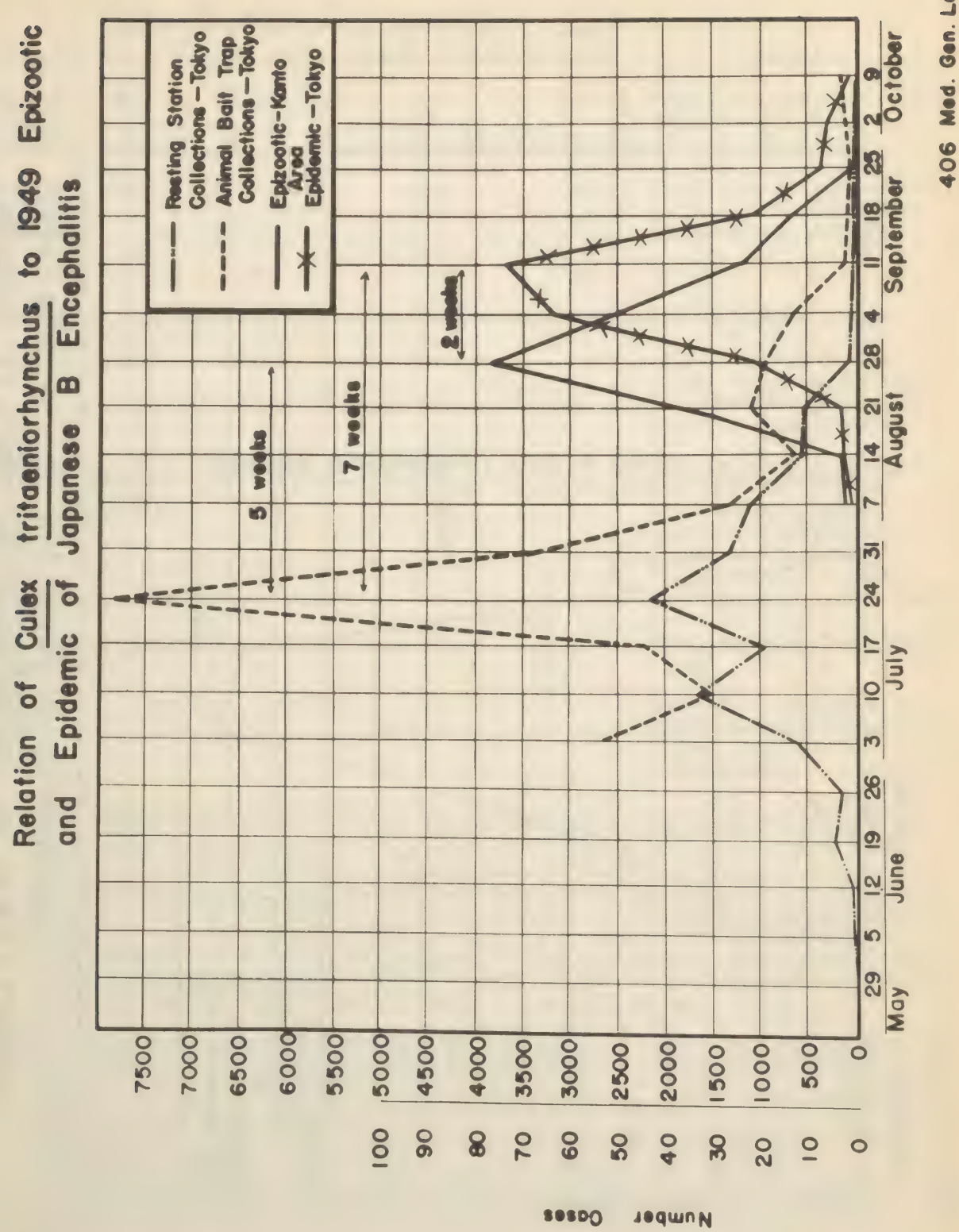
Relation of Culex tritaeniorhynchus to 1949 Epizootic and Epidemic of Japanese B Encephalitis
Resting Station Collections — Tokyo
Animal Bait Trap Collections — Tokyo
Epizootic-Kanto Area
Epidemic — Tokyo
5 weeks
7 weeks
2 weeks
Number Cases
May
June
July
August
September
October
29 5 12 19 26 3 10 17 24 31 7 14 21 28 4 11 18 25 2 9
0 10 20 30 40 50 60 70 80 90 100
0 500 1000 1500 2000 2500 3000 3500 4000 4500 5000 5500 6000 6500 7000 7500
406 Med. Gen. Lab.

Figure 10

Relation of Culex tritaeniorhynchus to 1948 Epizootic and Epidemic of Japanese B Encephalitis

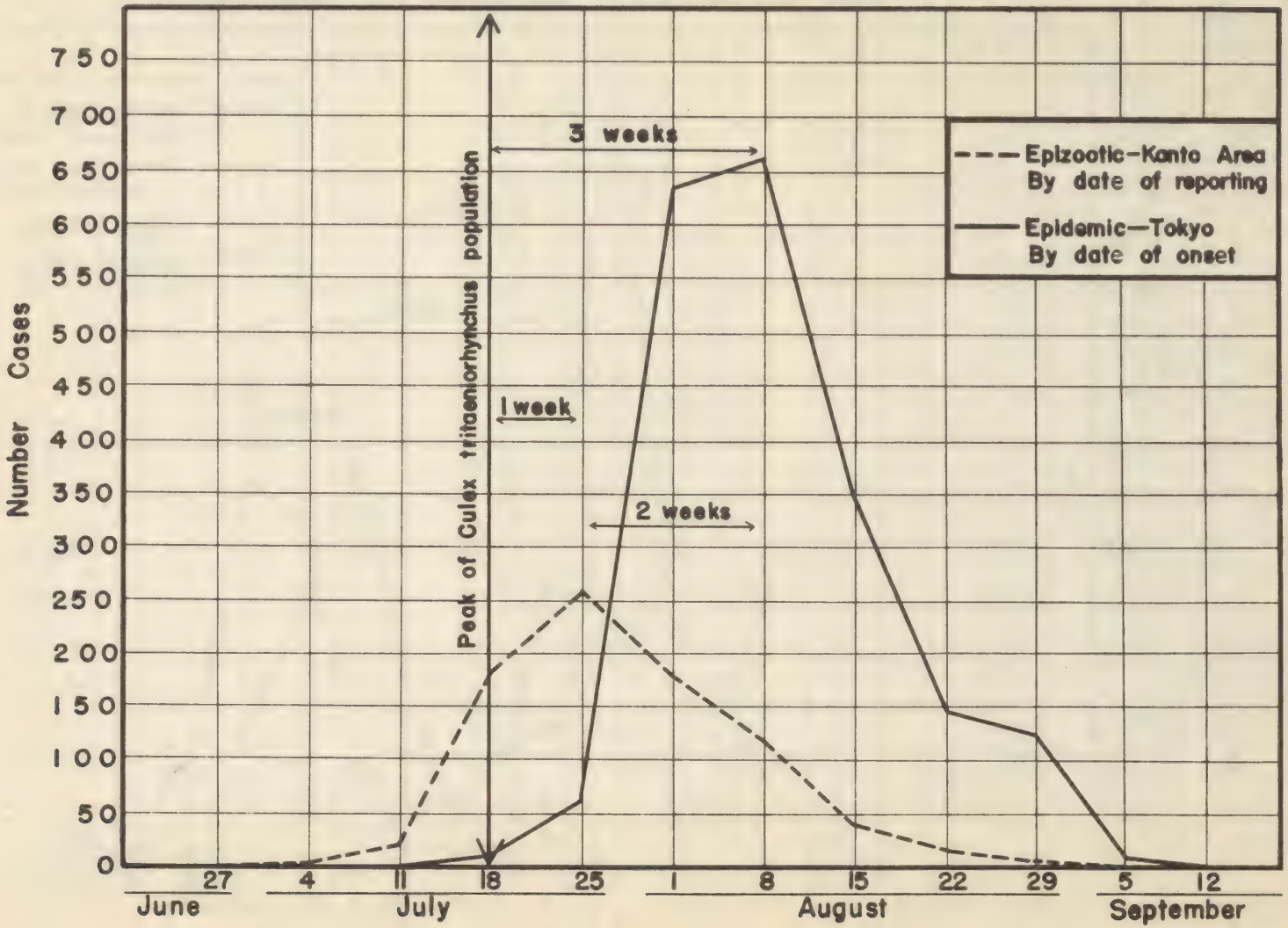

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Table XIV. Japanese Mosquito Species and Their Possible Importance as Vectors of Japanese B Encephalitis

Species	Human Attack Rate	Population level prior to epidemics	Virus Isolations from wild caught mosquitoes	Experimental vector	Remarks
Anopheles hyrcanus sinensis	High	High	Yes	Yes	Population curve with peaks. Strong vector suspect.
Armigeres subalbatus (obturbans)	High	Low-High	---	Yes	Population curve with peaks. Day and night biter. Strong vector suspect.
Aedes togoi	Low	Low	---	Yes	Possible vector.
A. japonicus	Very low	Low	---	Yes	Possible vector.
A. albopictus	High	Low-High	---	Yes	Localized breeder, short flight range, population level drops before epidemics. Possible vector.
A. flavopictus	High	Low-High	---	---	Same as for A. albopictus
A. vexans nipponii	High	High	---	Yes	Brood emerging species, with long flight range. Strong vector suspect.
Culex pipiens	Very high	Very high	Yes	Yes	Very important vector suspect.
Culex tritaeniorhynchus	Very high	Very high	Yes	Yes	Most important vector suspect.
C. quinquefasciatus	Very high	Very high	---	---	Limited to southern tip of Japan, but important on Okinawa. Very important vector suspect.

LaCasse (3) reports that some workers have been of the opinion that the sharp drop in the Culex tritaeniorhynchus population following the peak could be attributed to the seasonal drainage of rice paddies. Observations this year on rice paddy drainage and on larval collections, substantiate LaCasse's (3) contention that he could find no correlation between the drop in the Culex tritaeniorhynchus population and the drainage of rice paddies. Rice paddies were drained considerably after the rapid decline in the population of this species. The intensity of breeding in rice paddies was found to be very low for the species, although admittedly rice paddies cover extensive tracts in the Tokyo area.

Much has been written concerning the importance of summer heat as an epidemic determinant. Considerable importance has been attached to Mitamura's report (7) that the Japanese B Encephalitis virus will multiply most readily in the mosquito within the temperature range 27 to 31 C. (80.6 to 87.8°F.) During the fourteen days preceding the onset of the epidemic of 1948 the mean temperature was 77.3°F., while during the first fourteen days of the epidemic, encompassing the peak, the temperature averaged 80.2°F. Thus, during the pre-epidemic period and the first two weeks of the 1948 epidemic the temperature was below Mitamura's temperature range. By contrast the small 1949 epidemic saw a mean temperature of 81.9°F. during the two week pre-epidemic period, while the average temperature during the first two weeks of the epidemic (just prior to the epidemic peak) was 77.9°F.

Since the temperature during the heavy 1948 epidemic and the pre-epidemic period of that year fell below the range indicated by Mitamure, it is apparent that a broader temperature range must pertain. This broadened range is reached virtually every year in many areas of Japan, yet epidemics do not occur every year. Thus it is clear that the incidence of Japanese B Encephalitis is not determined solely by temperature, nor, as has been previously shown in this paper, is mosquito prevalence determined solely by temperature.

Statements are frequently made in the literature to the effect that the years of heaviest epidemics have been characterized by hot, dry summers (24), (8), (25). The months of July, August, and September of 1948 saw a total rainfall of 31.75 inches in Tokyo, while the same period in 1949 produced 20.29 inches of rainfall. It should be quite apparent that the occurrence of a hot, dry summer, such as that of 1949, is not necessarily beset by a heavy epidemic. The presence or absence of a large number of susceptibles in any given year should be of obvious importance. There is little data available to indicate the difference in mosquito species prevalence during hot, dry summers and cooler and wetter summers.

Summary - Basic facts have been obtained for 1949 regarding the prevalence of various mosquito species in the Tokyo area. Material was collected for demonstration of virus in mosquitoes naturally infected.

Suspected Dengue in Tokyo Area - Following the occurrence of a suspected case of dengue, a special two day mosquito survey was undertaken during August in the Grant Heights dependent housing area. Aedes aegypti could not be found at all, (this species is reportedly confined to the southernmost tip of the Japanese islands), nor could larvae or adults of Aedes albopictus be found in the confines of Grant Heights. However, five small larval collections of this latter species were taken in adjacent Japanese areas. The survey indicated that fear of a dengue epidemic was unwarranted.

Vectors of Scrub Typhus - Two trips of three days duration were taken to the Fuji Maneuver Areas to determine the occurrence and distribution of chigger vectors of scrub typhus. Several methods of collecting were utilized including: trapping of field rodents, placing laboratory rats as bait, running ground litter through a large Berlese Funnel, and attempting boot and card collections. Chiggers were taken in small numbers from trapped field rodents and from bait rats. Other collecting methods gave negative results. Three species of chiggers were taken in small numbers, none of which were Trombicula akamushi. These trips suggest that definition of the scrub typhus problem in the Fuji Maneuver area will require intensive and prolonged survey; since chigger populations appear to be sharply localized over an extensive area.

Bird collections - Personnel supplied from the Hooper Foundation, University of California, conducted a project to determine the possibility of birds or bird-mites being implicated in the epidemiology of JBE. Approximately 30,000 ectoparasites were collected from birds and nests. All were returned to the Hooper Foundation for identification and isolation attempts.

Special Rodent Survey - At the request of the Senior Medical Officer, Naval Forces, Far East, a special rodent survey was attempted. This was stimulated by the return of vessels previously loaned to a former ally. The heavy rodent infestation of these vessels were felt to constitute an opportunity to investigate possible disease reservoirs from a relatively inaccessible part of the world. Limitation of laboratory space prevented completion of satisfactory study. Results of study of 13 live black rats (Rattus rattus rattus) were negative in tests for certain viral, rickettsial, and bacterial disease.

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Administration

Burnett, Jack D., Major, MSC (Adjutant)
Teeter, Russell V., Captain, AGD (Inf) (Assistant Adjutant)
Wilson, Owen D., Captain, MSC (Supply Officer)
Roberts, Dolores M., GS-6 (Secretary)
Moore, Gladys, GS-4 (Stenographer)

Preventive Medicine Section

Simmons, Ingalls H., Major, MC

Medical Zoology Section

Hunter, George W. III, Colonel, MSC, Chief
Ritchie, Lawrence S., GS-12
Patterson, Katherine A., GS-7
Gumble, Adaline R., GS-7
Kimura, Betty N., GS-4

Serology Section

Stein, George J., GS-14, Chief
Hilton, Kathlyn C., Captain, WMSC

Chemistry Section

Stabile, Joseph N., Captain, MSC, Chief
Yamada, Mabel, GS-7

Bacteriology Section

Sanders, Arvey C., Major, MSC, Chief
Elrod, R. Perry, GS-12
Flintjer, John D., Captain, MSC
Recksiek, Margaret E., GS-6

Pathology Section

Aronson, Roland S., Lt. Colonel, MC, Chief
Scott, Edwin L., Captain, MC
Kleinerman, Jerome I., Captain, MC
Chambers, Wallace L., Captain, MC
Sullivan, Kathleen M., GS-4

Virus and Rickettsial Section

Burns, Kenneth F., Major, VC, Chief
Votaw, Floyd C., Major, VC
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Whatley, Lewis R., Captain, MC
Geib, Donna S., GS-7
Stokes, Julia C., GS-7

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